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(54) **METHODS FOR TREATING
INFLAMMATORY CONDITIONS USING
ANTIBODIES THAT BIND PAR-2**

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A61K 39/395 (2006.01)

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See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides compositions and methods relating to or derived from anti-PAR-2 antibodies. In particular embodiments, the invention provides antibodies that bind human PAR-2, PAR-2-binding fragments and derivatives of such antibodies, and PAR-2-binding polypeptides comprising such fragments. Other embodiments provide nucleic acids encoding such antibodies, antibody fragments and derivatives and polypeptides, cells comprising such polynucleotides, methods of making such antibodies, antibody fragments and derivatives and polypeptides, and methods of using such antibodies, antibody fragments and derivatives and polypeptides, including methods of treating or diagnosing subjects having PAR-2-related disorders or conditions.

5 Claims, 4 Drawing Sheets

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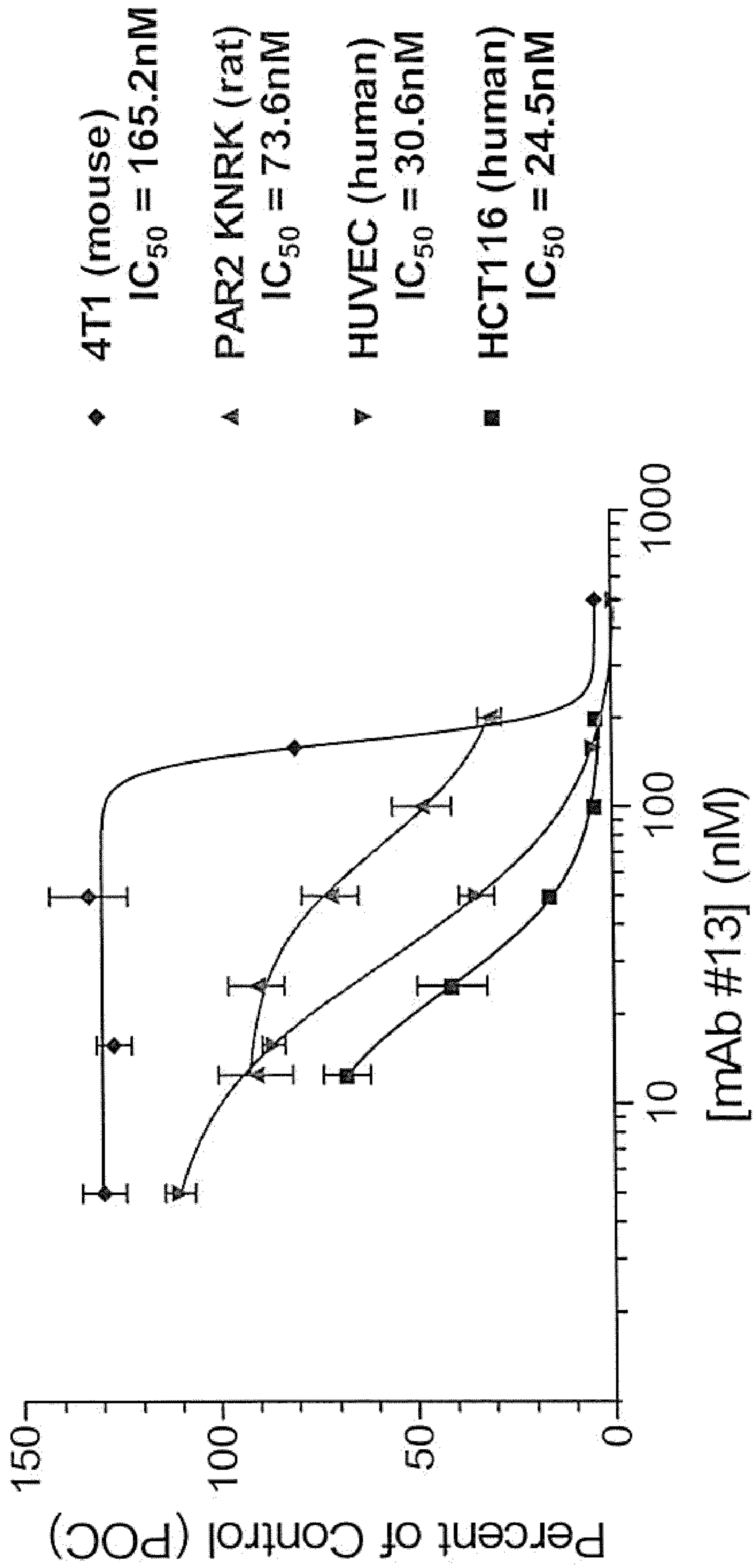


Figure 1

1° Antibody

α -hu16mer

SAM11

Clone 13-8

Clone 47-3

rAd#21

rAd#8

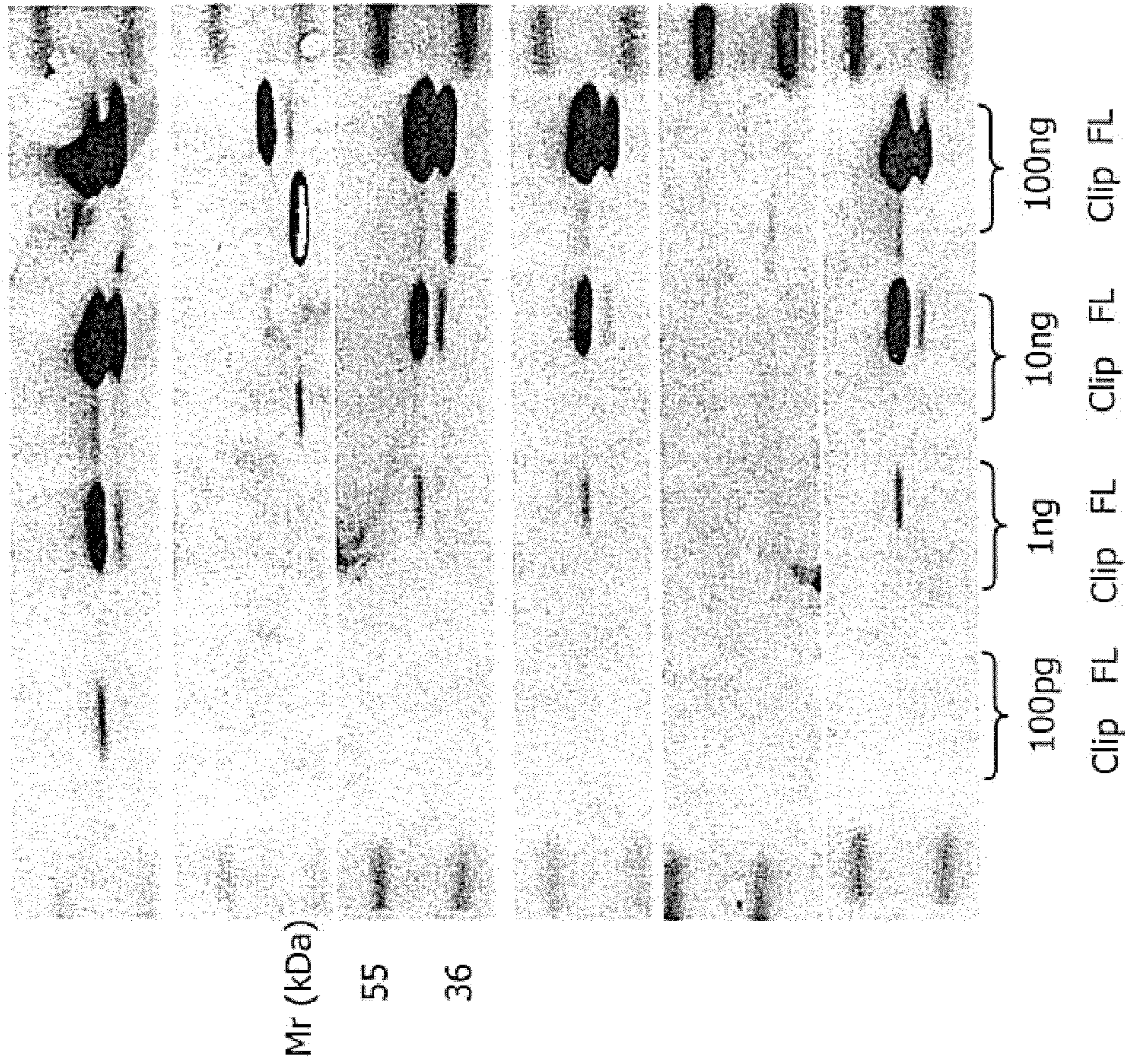


Figure 2

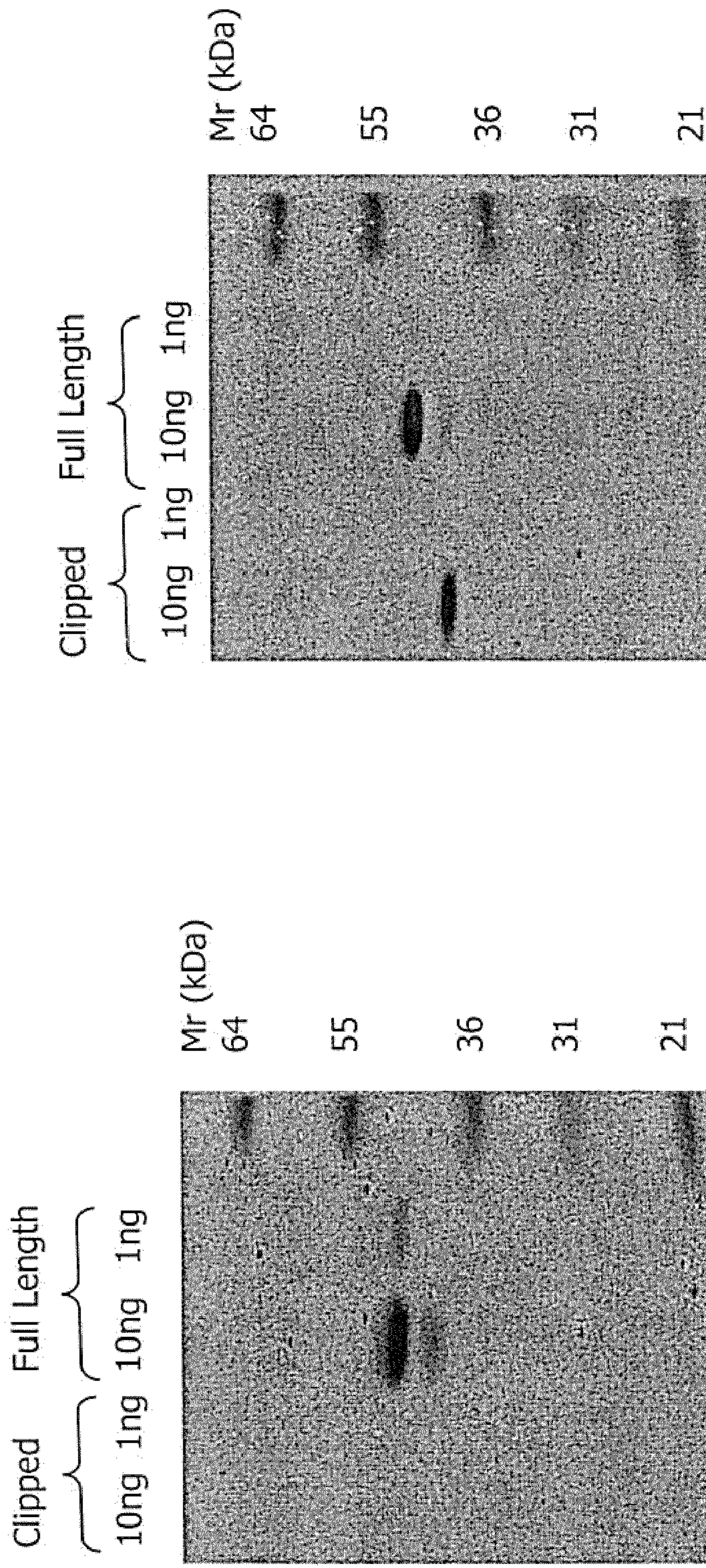


Figure 3

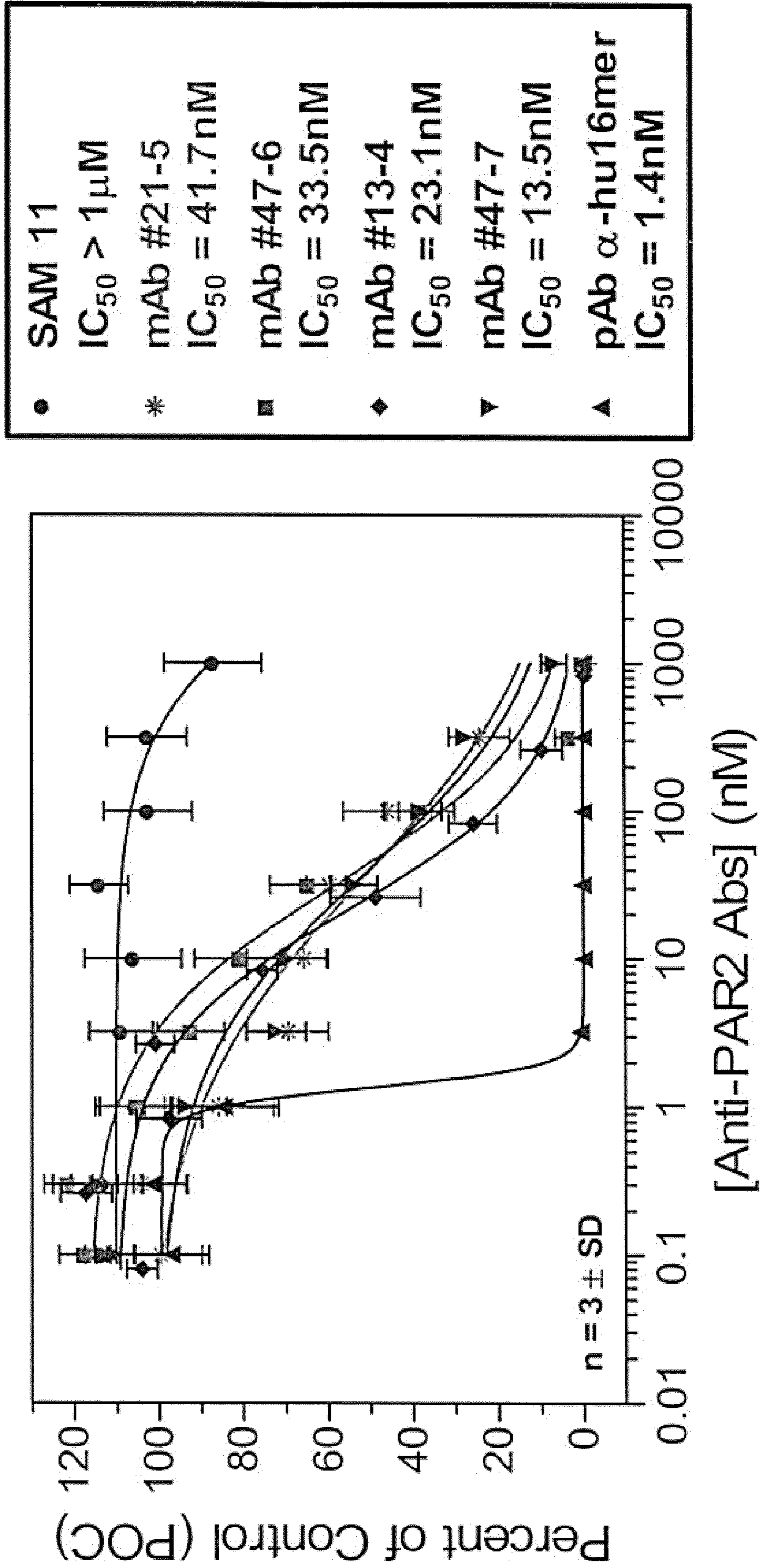


Figure 4

1

**METHODS FOR TREATING
INFLAMMATORY CONDITIONS USING
ANTIBODIES THAT BIND PAR-2**

CROSS-REFERENCE TO RELATED
APPLICATIONS

This application is a divisional of U.S. patent application Ser. No. 11/704,529, filed Feb. 8, 2007, now U.S. Pat. No. 7,888,482, which claims the benefit under 35 U.S.C. 119(e) of U.S. provisional application Ser. No. 60/772,456, filed Feb. 10, 2006; all of which are incorporated by reference herein.

REFERENCE TO SEQUENCE LISTING

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled A-1099-US-DIV_seq_listing.txt, created Jan. 18, 2011, which is 23.2 KB in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

This application provides compositions and methods relating to anti-PAR-2 antibodies.

BACKGROUND OF THE INVENTION

The Proteinase-activated receptor (PAR) family is a part of the seven-transmembrane G-coupled receptor superfamily. There are currently four known PARs, of which three (PARs-1, -3 and -4) are activated by thrombin; a fourth (PAR-2) is activated by trypsin or mast cell tryptase, but not by thrombin. PARs are widely distributed to a variety of tissues and participate in a number of physiological or pathophysiological phenomena such as platelet aggregation, inflammation and cardiovascular, digestive or respiratory functions.

PARs differ from other receptors in that activation is initiated by proteolytic cleavage of the N terminus of the PAR, which then forms a tethered ligand that interacts with the extracellular region (loop 2) of the same receptor polypeptide. Cleavage of PAR-2 occurs between the R and S residues of the protease cleavage domain, SKGRSLIG (amino acids 33 through 40 of SEQ ID NO:2), which is conserved between human, murine and rat PAR-2. Peptides that mimic the tethered ligand have been shown to have agonistic effects on PAR-2 (Saifeddine et al., *Br J Pharmacol* 118(3):521-30 [1996]; McGuire et al., *J Pharmacol Exp Ther* 309(3):1124-31 [2004]).

PAR-2 activates the G-protein-coupled receptor-mediated common signal transduction pathways, inositol 1,4,5-trisphosphate production and mobilization of Ca(2+), as well as multiple kinase pathways, including ERK, p38MAPK, JNK, and IKK. It is present on epithelial and endothelial cells, myocytes, fibroblasts, immune cells, neurons and glial cells in the kidney, pancreas, stomach, intestine, airway, skin, bladder and brain. The protease that activates PAR-2 is present during inflammation, and PAR-2 is upregulated by inflammatory factors such as tumour necrosis factor alpha, interleukin 1alpha and lipopolysaccharide. Moreover, studies utilizing PAR-2-deficient or-overexpressing mice confirm a role for this receptor in inflammation (Schmidlin et al., *J Immunol* 169, 5315-5321 [2002]; Ferrell et al., *J. Clin. Invest.* 111, 35-41 [2003]). Accordingly, there is a need in the art to

2

develop antagonists of PAR-2 activation, which will be useful in treating or ameliorating inflammatory conditions.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 demonstrates that ability of a PAR-2 antibody to antagonize PAR-2 activation in a FLIPR assay, using various PAR-2-expressing cells.

FIG. 2 provides a Western blot comparing the binding of various PAR-2 antibodies to full-length versus clipped PAR-2/Fc.

FIG. 3 presents Western blot results for an antagonistic PAR-2 antibody (clone 33) as well as an antibody that does not antagonize PAR-2 (clone 10).

FIG. 4 compares that ability of several PAR-2 antibodies to antagonize PAR-2 activation in a FLIPR assay.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides an isolated antigen binding protein that binds to proteinase activated receptor-2 (PAR-2). In another embodiment, the isolated antigen binding protein, when bound to a human PAR-2, inhibits proteolytic cleavage and/or subsequent signaling through said human PAR-2. In another embodiment, the isolated antigen binding protein inhibits proteolytic activation of PAR-2 by greater than about 80%. In another embodiment, the isolated antigen binding protein binds to full-length PAR-2 and binds to a lesser extent to cleaved PAR-2.

In another aspect of the invention, the isolated antigen binding protein specifically binds to the PAR-2 of a non-human primate, a cynomolgous monkey, a chimpanzee, a non-primate mammal, a rodent, a mouse, a rat, a hamster, a guinea pig, a cat, or a dog. In another embodiment, the isolated antigen binding protein comprises: a. a human antibody; b. a humanized antibody; c. a chimeric antibody; d. a monoclonal antibody; e. a monospecific antibody; f. a recombinant antibody; g. an antigen-binding antibody fragment; h. a single chain antibody; i. a diabody; j. a triabody; k. a tetrabody; l. a Fab fragment; m. a F(ab')₂ fragment; n. a domain antibody; o. an IgD antibody; p. an IgE antibody; q. an IgM antibody; r. an IgG1 antibody; s. an IgG2 antibody; t. an IgG3 antibody; u. an IgG4 antibody; or v. an IgG4 antibody having at least one mutation in a hinge region that alleviates a tendency to form intra-H chain disulfide bond.

In another aspect, the present invention provides an isolated cell that secretes an antigen binding protein that binds PAR-2. In another embodiment, the cell is a hybridoma. In another embodiment, the present invention provides a method of making an antigen binding protein that binds human PAR-2, comprising incubating said isolated cell under conditions that allow it to express said antigen binding protein.

In another aspect, the present invention provides a pharmaceutical composition comprising the antigen binding protein. In one embodiment, the present invention provides a method of treating a condition in a subject comprising administering to said subject said pharmaceutical composition, wherein said condition is treatable by reducing the activity of PAR-2 in said subject. In another embodiment, said subject is a human being. In another embodiment, said condition is an inflammatory condition of the skin, joints, gastrointestinal system and/or airway. In another embodiment, the method further comprises administering to said subject a second treatment. In another embodiment, said second treatment is administered to said subject before and/or simultaneously with and/or after said pharmaceutical composition is administered to said subject. In another embodiment, said second

treatment comprises an anti-inflammatory agent. In another embodiment, said second pharmaceutical composition comprises an agent selected from the group consisting of non-steroidal anti-inflammatory drugs, steroids, and immunomodulating agents. In another embodiment, said method comprises administering to said subject a third treatment.

In another aspect, the present invention provides a method of increasing the longevity of a subject comprising administering to said subject said pharmaceutical composition.

In another aspect, the present invention provides a method of decreasing PAR-2 activity in a subject in need thereof comprising administering to said subject said pharmaceutical composition.

In another aspect, the present invention provides a method of decreasing PAR-2 signaling in a subject in need thereof comprising administering to said subject said pharmaceutical composition.

In another aspect, the present invention provides a method of inhibiting the proteolytic activation of PAR-2 in a subject in need thereof comprising administering to said subject said pharmaceutical composition.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compositions, kits, and methods relating to molecules that bind to the Proteinase Activated Receptor 2 ("PAR-2"), including molecules that agonize or antagonize PAR-2, such as anti-PAR-2 antibodies, antibody fragments, and antibody derivatives, e.g., antagonistic anti-PAR-2 antibodies, antibody fragments, or antibody derivatives. Also provided are nucleic acids, and derivatives and fragments thereof, comprising a sequence of nucleotides that encodes all or a portion of a polypeptide that binds to PAR-2, e.g., a nucleic acid encoding all or part of an anti-PAR-2 antibody, antibody fragment, or antibody derivative, plasmids and vectors comprising such nucleic acids, and cells or cell lines comprising such nucleic acids and/or vectors and plasmids. The provided methods include, for example, methods of making, identifying, or isolating molecules that bind to PAR-2, such as anti-PAR-2 antibodies, methods of determining whether a molecule binds to PAR-2, methods of determining whether a molecule agonizes or antagonizes PAR-2, methods of making compositions, such as pharmaceutical compositions, comprising a molecule that binds to PAR-2, and methods for administering a molecule that binds PAR-2 to a subject, for example, methods for treating a condition mediated by PAR-2, and for agonizing or antagonizing a biological activity of PAR-2, in vivo or in vitro.

Polynucleotide and polypeptide sequences are indicated using standard one- or three-letter abbreviations. Unless otherwise indicated, each polypeptide sequence has amino termini at the left and a carboxy termini at the right; each single-stranded nucleic acid sequence, and the top strand of each double-stranded nucleic acid sequence, has a 5' termini at the left and a 3' termini at the right. A particular polypeptide or polynucleotide sequence also can be described by explaining how it differs from a reference sequence.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybrid-

ization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates (1992), and Harlow and Lane *Antibodies: A Laboratory Manual* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The terminology used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

The following terms, unless otherwise indicated, shall be understood to have the following meanings:

The term "isolated molecule" (where the molecule is, for example, a polypeptide, a polynucleotide, or an antibody) is a molecule that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or synthesized in a cellular system different from the cell from which it naturally originates, will be "isolated" from its naturally associated components. A molecule also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

The terms "PAR-2 inhibitor" and "PAR-2 antagonist" are used interchangeably. Each is a molecule that detectably inhibits at least one function of PAR-2. Conversely, a "PAR-2 agonist" is a molecule that detectably increases at least one function of PAR-2. The inhibition caused by a PAR-2 inhibitor need not be complete so long as it is detectable using an assay. Any assay of a function of PAR-2 can be used, examples of which are provided herein. Examples of functions of PAR-2 that can be inhibited by a PAR-2 inhibitor, or increased by a PAR-2 agonist, include protease-activated ligand binding, downstream signaling, and so on. Examples of types of PAR-2 inhibitors and PAR-2 agonists include, but are not limited to, PAR-2 binding polypeptides such as antigen binding proteins (e.g., PAR-2 inhibiting antigen binding proteins), antibodies, antibody fragments, and antibody derivatives.

The terms "peptide," "polypeptide" and "protein" each refers to a molecule comprising two or more amino acid residues joined to each other by peptide bonds. These terms encompass, e.g., native and artificial proteins, protein fragments and polypeptide analogs (such as muteins, variants, and fusion proteins) of a protein sequence as well as post-

translationally, or otherwise covalently or non-covalently, modified proteins. A peptide, polypeptide, or protein may be monomeric or polymeric.

The term “polypeptide fragment” as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion as compared to a corresponding full-length protein. Fragments can be, for example, at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 50, 70, 80, 90, 100, 150 or 200 amino acids in length. Fragments can also be, for example, at most 1,000, 750, 500, 250, 200, 175, 150, 125, 100, 90, 80, 70, 60, 50, 40, 30, 20, 15, 14, 13, 12, 11, or 10 amino acids in length. A fragment can further comprise, at either or both of its ends, one or more additional amino acids, for example, a sequence of amino acids from a different naturally-occurring protein (e.g., an Fc or leucine zipper domain) or an artificial amino acid sequence (e.g., an artificial linker sequence or a tag protein).

Polypeptides of the invention include polypeptides that have been modified in any way and for any reason, for example, to: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties. Analogs include muteins of a polypeptide. For example, single or multiple amino acid substitutions (e.g., conservative amino acid substitutions) may be made in the naturally occurring sequence (e.g., in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A “conservative amino acid substitution” is one that does not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterize the parent sequence or are necessary for its functionality). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. *Nature* 354:105 (1991), which are each incorporated herein by reference.

The present invention also provides non-peptide analogs of PAR-2 binding polypeptides. Non-peptide analogs are commonly used in the pharmaceutical industry as drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed “peptide mimetics” or “peptidomimetics,” see, for example, Fauchere, J. *Adv. Drug Res.* 15:29 (1986); Veber and Freidinger *TINS* p. 392 (1985); and Evans et al. *J. Med. Chem.* 30:1229 (1987), which are incorporated herein by reference. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a desired biochemical property or pharmacological activity), such as a human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: —CH₂NH—, —CH₂S—, —CH₂—CH₂—, —CH=CH-(cis and trans), —COCH₂—, —CH(OH)CH₂—, and —CH₂SO—, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may also be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by

Biochem. 61:387 (1992), incorporated herein by reference), for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

5 A “variant” of a polypeptide (e.g., an antibody) comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence. Variants of the invention include fusion proteins.

10 A “derivative” of a polypeptide is a polypeptide (e.g., an antibody) that has been chemically modified, e.g., via conjugation to another chemical moiety (such as, for example, polyethylene glycol or albumin, e.g., human serum albumin), phosphorylation, and glycosylation. Unless otherwise indicated, the term “antibody” includes, in addition to antibodies comprising two full-length heavy chains and two full-length light chains, derivatives, variants, fragments, and muteins thereof, examples of which are described below.

An “antigen binding protein” is a protein comprising a portion that binds to an antigen and, optionally, a scaffold or framework portion that allows the antigen binding portion to adopt a conformation that promotes binding of the antigen binding protein to the antigen. Examples of antigen binding proteins include antibodies, antibody fragments (e.g., an antigen binding portion of an antibody), antibody derivatives, and antibody analogs. The antigen binding protein can comprise, for example, an alternative protein scaffold or artificial scaffold with grafted CDRs or CDR derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced to, for example, stabilize the three-dimensional structure of the antigen binding protein as well as wholly synthetic scaffolds comprising, for example, a biocompatible polymer. See, for example, Korn-
dorfer et al., 2003, *Proteins: Structure, Function, and Bioinformatics*, Volume 53, Issue 1:121-129; Roque et al., 2004, *Biotechnol. Prog.* 20:639-654. In addition, peptide antibody mimetics (“PAMs”) can be used, as well as scaffolds based on antibody mimetics utilizing fibronectin components as a scaffold.

40 An antigen binding protein can have, for example, the structure of a naturally occurring immunoglobulin. An “immunoglobulin” is a tetrameric molecule. In a naturally occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kDa) and one “heavy” chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody’s isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a “J” region of about 12 or more amino acids, with the heavy chain also including a “D” region of about 10 more amino acids. See generally, *Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites.

65 Naturally occurring immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. From N-terminus to C-terminus, both light and heavy chains com-

prise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat et al. in *Sequences of Proteins of Immunological Interest*, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991.

Naturally occurring antibodies can be obtained from sources such as serum or plasma that contain immunoglobulins having varied antigenic specificity. If such antibodies are subjected to affinity purification, they can be enriched for a particular antigenic specificity. Such enriched preparations of antibodies usually are made of less than about 10% antibody having specific binding activity for the particular antigen. Antibodies prepared in this manner are often referred to as “monospecific.”

An “antibody” refers to an intact immunoglobulin or to an antigen binding portion thereof that competes with the intact antibody for specific binding, unless otherwise specified. Antigen binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen binding portions include, inter alia, Fab, Fab', F(ab')₂, Fv, domain antibodies (dAbs), and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies, triabodies, tetrabodies, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide.

A Fab fragment is a monovalent fragment having the V_L, V_H, C_L and C_H1 domains; a F(ab')₂ fragment is a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment has the V_H and C_H1 domains; an Fv fragment has the V_L and V_H domains of a single arm of an antibody; and a dAb fragment has a V_H domain, a V_L domain, or an antigen-binding fragment of a V_H or V_L domain (U.S. Pat. Nos. 6,846,634, 6,696,245, US App. Pub. No. 05/0202512, 04/0202995, 04/0038291, 04/0009507, 03/0039958, Ward et al., Nature 341:544-546, 1989).

A single-chain antibody (scFv) is an antibody in which a V_L and a V_H region are joined via a linker (e.g., a synthetic sequence of amino acid residues) to form a continuous protein chain wherein the linker is long enough to allow the protein chain to fold back on itself and form a monovalent antigen binding site (see, e.g., Bird et al., 1988, Science 242:423-26 and Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-83). Diabodies are bivalent antibodies comprising two polypeptide chains, wherein each polypeptide chain comprises V_H and V_L domains joined by a linker that is too short to allow for pairing between two domains on the same chain, thus allowing each domain to pair with a complementary domain on another polypeptide chain (see, e.g., Holliger et al., 1993, Proc. Natl. Acad. Sci. USA 90:6444-48, and Poljak et al., 1994, Structure 2:1121-23). If the two polypeptide chains of a diabody are identical, then a diabody resulting from their pairing will have two identical antigen binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen binding sites. Similarly, triabodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen binding sites, respectively, which can be the same or different.

Complementarity determining regions (CDRs) and framework regions (FR) of a given antibody may be identified using the system described by Kabat et al. in *Sequences of Proteins of Immunological Interest*, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991. One or more CDRs may be incorporated into a mol-

ecule either covalently or noncovalently to make it an antigen binding protein. An antigen binding protein may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the antigen binding protein to specifically bind to a particular antigen of interest.

An antigen binding protein may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For example, a naturally occurring human immunoglobulin typically has two identical binding sites, while a “bispecific” or “bifunctional” antibody has two different binding sites.

The term “human antibody” includes all antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In one embodiment, all of the variable and constant domains are derived from human immunoglobulin sequences (a fully human antibody). These antibodies may be prepared in a variety of ways, examples of which are described below, including through the immunization with an antigen of interest of a mouse that is genetically modified to express antibodies derived from human heavy and/or light chain-encoding genes.

A humanized antibody has a sequence that differs from the sequence of an antibody derived from a non-human species by one or more amino acid substitutions, deletions, and/or additions, such that the humanized antibody is less likely to induce an immune response, and/or induces a less severe immune response, as compared to the non-human species antibody, when it is administered to a human subject. In one embodiment, certain amino acids in the framework and constant domains of the heavy and/or light chains of the non-human species antibody are mutated to produce the humanized antibody. In another embodiment, the constant domain(s) from a human antibody are fused to the variable domain(s) of a non-human species. In another embodiment, one or more amino acid residues in one or more CDR sequences of a non-human antibody are changed to reduce the likely immunogenicity of the non-human antibody when it is administered to a human subject, wherein the changed amino acid residues either are not critical for immunospecific binding of the antibody to its antigen, or the changes to the amino acid sequence that are made are conservative changes, such that the binding of the humanized antibody to the antigen is not significantly worse than the binding of the non-human antibody to the antigen. Examples of how to make humanized antibodies may be found in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293.

The term “chimeric antibody” refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies. In one embodiment, one or more of the CDRs are derived from a human anti-PAR-2 antibody. In another embodiment, all of the CDRs are derived from a human anti-PAR-2 antibody. In another embodiment, the CDRs from more than one human anti-PAR-2 antibodies are mixed and matched in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti-PAR-2 antibody, a CDR2 and a CDR3 from the light chain of a second human anti-PAR-2 antibody, and the CDRs from the heavy chain from a third anti-PAR-2 antibody. Further, the framework regions may be derived from one of the same anti-PAR-2 antibodies, from one or more different antibodies, such as a human antibody, or from a humanized antibody. In one example of a chimeric antibody, a portion of the heavy and/or light chain is identical with, homologous to, or derived from an antibody from a particular species or belonging to a

particular antibody class or subclass, while the remainder of the chain(s) is/are identical with, homologous to, or derived from an antibody (-ies) from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies that exhibit the desired biological activity (i.e., the ability to specifically bind PAR-2). See, e.g., U.S. Pat. No. 4,816,567 and Morrison, 1985, Science 229: 1202-07.

A "neutralizing antibody" or an "inhibitory antibody" is an antibody that inhibits the proteolytic activation of PAR-2 when an excess of the anti-PAR-2 antibody reduces the amount of activation by at least about 20% using an assay such as those described herein in the Examples. In various embodiments, the antigen binding protein reduces the amount of amount of proteolytic activation of PAR-2 by at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, and 99.9%.

Fragments or analogs of antibodies can be readily prepared by those of ordinary skill in the art following the teachings of this specification and using techniques well-known in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. See, e.g., Bowie et al., 1991, Science 253:164.

A "CDR grafted antibody" is an antibody comprising one or more CDRs derived from an antibody of a particular species or isotype and the framework of another antibody of the same or different species or isotype.

A "multi-specific antibody" is an antibody that recognizes more than one epitope on one or more antigens. A subclass of this type of antibody is a "bi-specific antibody" which recognizes two distinct epitopes on the same or different antigens.

An antigen binding protein "specifically binds" to an antigen (e.g., human PAR-2) if it binds to the antigen with a dissociation constant of 1 nanomolar or less.

An "antigen binding domain," "antigen binding region," or "antigen binding site" is a portion of an antigen binding protein that contains amino acid residues (or other moieties) that interact with an antigen and contribute to the antigen binding protein's specificity and affinity for the antigen. For an antibody that specifically binds to its antigen, this will include at least part of at least one of its CDR domains.

An "epitope" is the portion of a molecule that is bound by an antigen binding protein (e.g., by an antibody). An epitope can comprise non-contiguous portions of the molecule (e.g., in a polypeptide, amino acid residues that are not contiguous in the polypeptide's primary sequence but that, in the context of the polypeptide's tertiary and quaternary structure, are near enough to each other to be bound by an antigen binding protein).

The "percent identity" of two polynucleotide or two polypeptide sequences is determined by comparing the sequences using the GAP computer program (a part of the GCG Wisconsin Package, version 10.3 (Accelrys, San Diego, Calif.)) using its default parameters.

The terms "polynucleotide," "oligonucleotide" and "nucleic acid" are used interchangeably throughout and include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogs of the DNA or RNA generated using nucleotide analogs (e.g., peptide nucleic acids and non-naturally occurring nucleotide analogs), and hybrids

thereof. The nucleic acid molecule can be single-stranded or double-stranded. In one embodiment, the nucleic acid molecules of the invention comprise a contiguous open reading frame encoding an antibody, or a fragment, derivative, mutein, or variant thereof, of the invention.

Two single-stranded polynucleotides are "the complement" of each other if their sequences can be aligned in an anti-parallel orientation such that every nucleotide in one polynucleotide is opposite its complementary nucleotide in the other polynucleotide, without the introduction of gaps, and without unpaired nucleotides at the 5' or the 3' end of either sequence. A polynucleotide is "complementary" to another polynucleotide if the two polynucleotides can hybridize to one another under moderately stringent conditions. Thus, a polynucleotide can be complementary to another polynucleotide without being its complement.

A "vector" is a nucleic acid that can be used to introduce another nucleic acid linked to it into a cell. One type of vector is a "plasmid," which refers to a linear or circular double stranded DNA molecule into which additional nucleic acid segments can be ligated. Another type of vector is a viral vector (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), wherein additional DNA segments can be introduced into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors comprising a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. An "expression vector" is a type of vector that can direct the expression of a chosen polynucleotide.

A nucleotide sequence is "operably linked" to a regulatory sequence if the regulatory sequence affects the expression (e.g., the level, timing, or location of expression) of the nucleotide sequence. A "regulatory sequence" is a nucleic acid that affects the expression (e.g., the level, timing, or location of expression) of a nucleic acid to which it is operably linked. The regulatory sequence can, for example, exert its effects directly on the regulated nucleic acid, or through the action of one or more other molecules (e.g., polypeptides that bind to the regulatory sequence and/or the nucleic acid). Examples of regulatory sequences include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Further examples of regulatory sequences are described in, for example, Goeddel, 1990, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. and Baron et al., 1995, Nucleic Acids Res. 23:3605-06.

A "host cell" is a cell that can be used to express a nucleic acid, e.g., a nucleic acid of the invention. A host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote, for example, a single-celled eukaryote (e.g., a yeast or other fungus), a plant cell (e.g., a tobacco or tomato plant cell), an animal cell (e.g., a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Examples of host cells include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (see Gluzman et al., 1981, Cell 23:175), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (see Rasmussen et al., 1998, Cytotechnology 28:31) or CHO strain DX-B11, which is deficient in DHFR (see Urlaub et al., 1980, Proc. Natl. Acad. Sci. USA 77:4216-20), HeLa cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (see McMahan et al., 1991,

EMBO J. 10:2821), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase “recombinant host cell” can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, e.g., mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

PAR-2

As discussed previously, PAR-2 is member of the seven-transmembrane G-coupled receptor superfamily; activation is initiated by proteolytic cleavage of the N terminus to form a tethered ligand. The nucleotide and amino acid sequences of human PAR-2 are shown in SEQ ID NOs:1 and 2; the amino acid sequence of mouse PAR-2 is shown in SEQ ID NO:3 and that of rat PAR-2 is shown in SEQ ID NO:4. Proteolytic cleavage yields the active form of this receptor, which is referred to interchangeably herein as “cleaved” or “clipped” PAR-2.

Antigen Binding Proteins

In one aspect, the present invention provides antigen binding proteins (e.g., antibodies, antibody fragments, antibody derivatives, antibody muteins, and antibody variants) that bind to PAR-2, e.g., human PAR-2.

Antigen binding proteins in accordance with the present invention include antigen binding proteins that inhibit a biological activity of PAR-2. Examples of such biological activities include activation of G-protein-coupled receptor-mediated common signal transduction pathways such as inositol 1,4,5-trisphosphate production and mobilization of Ca(2+), and activation of multiple kinase pathways, including ERK, p38MAPK, JNK, and IKK. Other biological activities include those mediated by PAR-2 in vivo, such as the response to trauma and inflammation; in particular, PAR-2 is involved in the cardiovascular, pulmonary and gastrointestinal systems, where it controls inflammation and nociception (perception of pain). PAR-2 activation also plays a role in the inflammatory response, chronic activation of which can lead to disease conditions.

Different antigen binding proteins may bind to different domains or epitopes of PAR-2 or act by different mechanisms of action. Examples include but are not limited to antigen binding proteins that interfere with proteolytic activation of PAR-2 or that inhibit signal transduction. The site of action may be, for example, intracellular (e.g., by interfering with an intracellular signaling cascade) or extracellular. An antigen binding protein need not completely inhibit PAR-2 induced activity to find use in the present invention; rather, antigen binding proteins that reduce a particular activity of PAR-2 are contemplated for use as well. (Discussions herein of particular mechanisms of action for PAR-2-binding antigen binding proteins in treating particular diseases are illustrative only, and the methods presented herein are not bound thereby.)

Other derivatives of anti-PAR-2 antibodies within the scope of this invention include covalent or aggregative conjugates of anti-PAR-2 antibodies, or fragments thereof, with other proteins or polypeptides, such as by expression of recombinant fusion proteins comprising heterologous polypeptides fused to the N-terminus or C-terminus of an anti-PAR-2 antibody polypeptide. For example, the conjugated peptide may be a heterologous signal (or leader) polypeptide, e.g., the yeast alpha-factor leader, or a peptide such as an epitope tag. Antigen binding protein-containing fusion proteins can comprise peptides added to facilitate purification or identification of antigen binding protein (e.g., poly-His). An antigen binding protein also can be linked to the FLAG peptide Asp-Tyr-Lys-Asp-Asp-Asp-Lys (DYKDDDDK) (SEQ ID NO:7) as described in Hopp et al., *Bio/Technology* 6:1204, 1988, and U.S. Pat. No. 5,011,912. The FLAG peptide is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody (mAb), enabling rapid assay and facile purification of expressed recombinant protein. Reagents useful for preparing fusion proteins in which the FLAG peptide is fused to a given polypeptide are commercially available (Sigma, St. Louis, Mo.).

Oligomers that contain one or more antigen binding proteins may be employed as PAR-2 antagonists. Oligomers may be in the form of covalently-linked or non-covalently-linked dimers, trimers, or higher oligomers. Oligomers comprising two or more antigen binding protein are contemplated for use, with one example being a homodimer. Other oligomers include heterodimers, homotrimers, heterotrimers, homotetramers, heterotetramers, etc.

One embodiment is directed to oligomers comprising multiple antigen binding proteins joined via covalent or non-covalent interactions between peptide moieties fused to the antigen binding proteins. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of antigen binding proteins attached thereto, as described in more detail below.

In particular embodiments, the oligomers comprise from two to four antigen binding proteins. The antigen binding proteins of the oligomer may be in any form, such as any of the forms described above, e.g., variants or fragments. Preferably, the oligomers comprise antigen binding proteins that have PAR-2 binding activity.

In one embodiment, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, e.g., by Ashkenazi et al., 1991, PNAS USA 88:10535; Byrn et al., 1990, Nature 344:677; and Hollenbaugh et al., 1992 “Construction of Immunoglobulin Fusion Proteins”, in *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1-10.19.11.

One embodiment of the present invention is directed to a dimer comprising two fusion proteins created by fusing a PAR-2 binding fragment of an anti-PAR-2 antibody to the Fc region of an antibody. The dimer can be made by, for example, inserting a gene fusion encoding the fusion protein into an appropriate expression vector, expressing the gene fusion in host cells transformed with the recombinant expression vector, and allowing the expressed fusion protein to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield the dimer.

The term “Fc polypeptide” as used herein includes native and mutein forms of polypeptides derived from the Fc region

of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization also are included. Fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

One suitable Fc polypeptide, described in PCT application WO 93/10151 (hereby incorporated by reference), is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Pat. No. 5,457,035 and in Baum et al., 1994, EMBO J. 13:3992-4001. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors.

In other embodiments, the variable portion of the heavy and/or light chains of an anti-PAR-2 antibody may be substituted for the variable portion of an antibody heavy and/or light chain.

Alternatively, the oligomer is a fusion protein comprising multiple antigen binding proteins, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Pat. Nos. 4,751,180 and 4,935,233.

Another method for preparing oligomeric antigen binding proteins involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., 1988, Science 240:1759), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al., 1994, FEBS Letters 344:191, hereby incorporated by reference. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow et al., 1994, Semin. Immunol 6:267-78. In one approach, recombinant fusion proteins comprising an anti-PAR-2 antibody fragment or derivative fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomeric anti-PAR-2 antibody fragments or derivatives that form are recovered from the culture supernatant.

In one aspect, the present invention provides antigen binding proteins that interfere with the proteolytic activation of a PAR-2. Such antigen binding proteins can be made against PAR-2, or a fragment, variant or derivative thereof, and screened in conventional assays for the ability to interfere with proteolytic activation of PAR-2. Examples of suitable assays are assays that test the antigen binding proteins for the ability to inhibit proteolytic activation of cells expressing PAR-2, or that test antigen binding proteins for the ability to reduce a biological or cellular response that results from the proteolytic activation of cell surface PAR-2 receptors. Additional assays that test the antigen binding proteins include those that qualitatively or quantitatively compare the binding of an antigen binding protein to a full-length, mature PAR-2 polypeptide to the binding of a proteolytically cleaved PAR-2 polypeptide, several examples of which are disclosed herein.

In another aspect, the present invention provides an antigen binding protein that demonstrates species selectivity. In one embodiment, the antigen binding protein binds to one or more mammalian PAR-2, for example, to human PAR-2 and one or more of mouse, rat, guinea pig, hamster, gerbil, cat, rabbit, dog, goat, sheep, cow, horse, camel, and non-human primate PAR-2. In another embodiment, the antigen binding protein binds to one or more primate PAR-2, for example, to human PAR-2 and one or more of cynomologous, marmoset, rhesus, and chimpanzee PAR-2. In another embodiment, the antigen binding protein binds specifically to human, cynomologous, marmoset, rhesus, or chimpanzee PAR-2. In another embodiment, the antigen binding protein does not bind to one or more of mouse, rat, guinea pig, hamster, gerbil, cat, rabbit, dog, goat, sheep, cow, horse, camel, and non-human primate PAR-2. In another embodiment, the antigen binding protein does not bind to a New World monkey species such as a marmoset. In another embodiment, the antigen binding protein does not exhibit specific binding to any naturally occurring protein other than PAR-2. In another embodiment, the antigen binding protein does not exhibit specific binding to any naturally occurring protein other than mammalian PAR-2. In another embodiment, the antigen binding protein does not exhibit specific binding to any naturally occurring protein other than primate PAR-2. In another embodiment, the antigen binding protein does not exhibit specific binding to any naturally occurring protein other than human PAR-2. In another embodiment, the antigen binding protein specifically binds to mouse, rat, cynomolgus monkey, and human PAR-2. In another embodiment, the antigen binding protein specifically binds to mouse, rat, cynomolgus monkey, and human PAR-2 with a similar binding affinity. In another embodiment, the antigen binding protein blocks binding of proteolytic activation of mouse, rat, cynomolgus monkey, and human PAR-2. In another embodiment, the antigen binding protein has a similar IC_{50} against mouse, rat, cynomolgus monkey, and human PAR-2 in a Ca^{2+} mobilization assay.

One may determine the selectivity of an antigen binding protein for a PAR-2 using methods well known in the art and following the teachings of the specification. For example, one may determine the selectivity using Western blot, FACS, ELISA or RIA.

In another aspect, the present invention provides a PAR-2 binding antigen binding protein (for example, an anti-PAR-2 antibody), that has one or more of the following characteristics: binds to both human and murine PAR-2, inhibits the proteolytic activation of human PAR-2, inhibits the proteolytic activation of murine PAR-2, binds to or near the proteolytic cleavage site of PAR-2, causes relatively little down-regulation of cell-surface expressed PAR-2.

Antigen-binding fragments of antigen binding proteins of the invention may be produced by conventional techniques. Examples of such fragments include, but are not limited to, Fab and $F(ab')_2$ fragments. Antibody fragments and derivatives produced by genetic engineering techniques also are contemplated.

Additional embodiments include chimeric antibodies, e.g., humanized versions of non-human (e.g., murine) monoclonal antibodies. Such humanized antibodies may be prepared by known techniques, and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable domain of a murine antibody (or all or part of the antigen binding site thereof) and a constant domain derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable domain fragment

(lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al., 1988, Nature 332:323, Liu et al., 1987, Proc. Nat. Acad. Sci. USA 84:3439, Larrick et al., 1989, Bio/Technology 7:934, and Winter et al., 1993, TIPS 14:139. In one embodiment, the chimeric antibody is a CDR grafted antibody. Techniques for humanizing antibodies are discussed in, e.g., U.S. patent application Ser. No. 10/194,975 (published Feb. 27, 2003), U.S. Pat. Nos. 5,869,619, 5,225, 539, 5,821,337, 5,859,205, Padlan et al., 1995, FASEB J. 9:133-39, and Tamura et al., 2000, J. Immunol 164:1432-41.

Procedures have been developed for generating human or partially human antibodies in non-human animals. For example, mice in which one or more endogenous immunoglobulin genes have been inactivated by various means have been prepared. Human immunoglobulin genes have been introduced into the mice to replace the inactivated mouse genes. Antibodies produced in the animal incorporate human immunoglobulin polypeptide chains encoded by the human genetic material introduced into the animal. In one embodiment, a non-human animal, such as a transgenic mouse, is immunized with a PAR-2 polypeptide, such that antibodies directed against the PAR-2 polypeptide are generated in the animal. One example of a suitable immunogen is a soluble human PAR-2, such as a polypeptide comprising the proteolytic cleavage site of PAR-2, or other immunogenic fragment PAR-2. Another example of a suitable immunogen is cells expressing high levels of PAR-2, or cell membrane preparations therefrom. Examples of techniques for production and use of transgenic animals for the production of human or partially human antibodies are described in U.S. Pat. Nos. 5,814,318, 5,569,825, and 5,545,806, Davis et al., 2003, *Production of human antibodies from transgenic mice* in Lo, ed. Antibody Engineering: Methods and Protocols, Humana Press, NJ:191-200, Kellermann et al., 2002, Curr Opin Biotechnol. 13:593-97, Russel et al., 2000, Infect Immun. 68:1820-26, Gallo et al., 2000, Eur J Immun. 30:534-40, Davis et al., 1999, Cancer Metastasis Rev. 18:421-25, Green, 1999, J Immunol Methods. 231:11-23, Jakobovits, 1998, Advanced Drug Delivery Reviews 31:33-42, Green et al., 1998, J Exp Med. 188:483-95, Jakobovits A, 1998, Exp. Opin. Invest. Drugs. 7:607-14, Tsuda et al., 1997, Genomics. 42:413-21, Mendez et al., 1997, Nat Genet. 15:146-56, Jakobovits, 1994, Curr Biol. 4:761-63, Arbones et al., 1994, Immunity 1.247-60, Green et al., 1994, Nat Genet. 7:13-21, Jakobovits et al., 1993, Nature. 362:255-58, Jakobovits et al., 1993, Proc Natl Acad Sci USA. 90:2551-55. Chen, J., M. Trounstein, F. W. Alt, F. Young, C. Kurahara, J. Loring, D. Huszar "Immunoglobulin gene rearrangement in B cell deficient mice generated by targeted deletion of the JH locus." International Immunology 5 (1993): 647-656, Choi et al., 1993, Nature Genetics 4: 117-23, Fishwild et al., 1996, Nature Biotechnology 14: 845-51, Harding et al., 1995, Annals of the New York Academy of Sciences, Lonberg et al., 1994, Nature 368: 856-59, Lonberg, 1994, *Transgenic Approaches to Human Monoclonal Antibodies* in Handbook of Experimental Pharmacology 113: 49-101, Lonberg et al., 1995, Internal Review of Immunology 13: 65-93, Neuberger, 1996, Nature Biotechnology 14: 826, Taylor et al., 1992, Nucleic Acids Research 20: 6287-95, Taylor et al., 1994, International Immunology 6: 579-91, Tomizuka et al., 1997, Nature Genetics 16: 133-43, Tomizuka et al., 2000, Proceedings of the National Academy of Sciences USA 97: 722-27, Tuaille et al., 1993, Proceedings of the National Academy of Sciences USA 90: 3720-24, and Tuaille et al., 1994, Journal of Immunology 152: 2912-20.

In another aspect, the present invention provides monoclonal antibodies that bind to PAR-2. Monoclonal antibodies may be produced using any technique known in the art, e.g., by immortalizing spleen cells harvested from the transgenic animal after completion of the immunization schedule. The spleen cells can be immortalized using any technique known in the art, e.g., by fusing them with myeloma cells to produce hybridomas. Myeloma cells for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). Examples of suitable cell lines for use in mouse fusions include Sp-20, P3-X63/Ag8, P3-X63-Ag8.653, NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; examples of cell lines used in rat fusions include R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210. Other cell lines useful for cell fusions are U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6.

In one embodiment, a hybridoma cell line is produced by immunizing an animal (e.g., a transgenic animal having human immunoglobulin sequences) with a PAR-2 immunogen; harvesting spleen cells from the immunized animal; fusing the harvested spleen cells to a myeloma cell line, thereby generating hybridoma cells; establishing hybridoma cell lines from the hybridoma cells, and identifying a hybridoma cell line that produces an antibody that binds a PAR-2 polypeptide. Such hybridoma cell lines, and anti-PAR-2 monoclonal antibodies produced by them, are encompassed by the present invention.

Monoclonal antibodies secreted by a hybridoma cell line can be purified using any technique known in the art. Hybridomas or mAbs may be further screened to identify mAbs with particular properties, such as the ability to block a PAR-2 induced activity. Examples of such screens are provided in the examples below.

Monoclonal antibodies can also be produced using a process referred to as genetic immunization. For example, a nucleic acid encoding the antigen of interest can be incorporated into a viral vector (such as an adenoviral vector). The resulting vector is then used to develop an immune response against the antigen of interest in a suitable host animal (for example, a non-obese diabetic, or NOD, mouse). This technique is substantially described by Ritter et al., Biodrugs 16(1): 3-10 (2002), the disclosure of which is incorporated by reference herein.

Molecular evolution of the complementarity determining regions (CDRs) in the center of the antibody binding site also has been used to isolate antibodies with increased affinity, for example, antibodies having increased affinity for c-erbB-2, as described by Schier et al., 1996, J. Mol. Biol. 263:551. Accordingly, such techniques are useful in preparing antibodies to PAR-2.

Antigen binding proteins directed against a PAR-2 can be used, for example, in assays to detect the presence of PAR-2 polypeptides, either in vitro or in vivo. The antigen binding proteins also may be employed in purifying PAR-2 proteins by immunoaffinity chromatography. Those antigen binding proteins that additionally can block proteolytic activation of PAR-2 may be used to inhibit a biological activity that results from such binding. Blocking antigen binding proteins can be used in the methods of the present invention. Such antigen binding proteins that function as PAR-2 antagonists may be employed in treating any PAR-2-induced condition, including but not limited to inflammatory conditions. In one embodiment, a human anti-PAR-2 monoclonal antibody gen-

erated by procedures involving immunization of transgenic mice is employed in treating such conditions.

Antigen binding proteins may be employed in an in vitro procedure, or administered in vivo to inhibit a PAR-2-induced biological activity. Disorders caused or exacerbated (directly or indirectly) by the proteolytic activation of PAR-2, examples of which are provided herein, thus may be treated. In one embodiment, the present invention provides a therapeutic method comprising in vivo administration of a PAR-2 blocking antigen binding protein to a mammal in need thereof in an amount effective for reducing a PAR-2-induced biological activity.

Antigen binding proteins of the invention include partially human and fully human monoclonal antibodies that inhibit a biological activity of PAR-2. One embodiment is directed to a human monoclonal antibody that at least partially blocks proteolytic activation of human PAR-2. In one embodiment, the antibodies are generated by immunizing a transgenic mouse with a PAR-2 immunogen. In another embodiment, the immunogen is a human PAR-2 polypeptide (e.g., a soluble fragment comprising all or part of the PAR-2 cleavage site). Hybridoma cell lines derived from such immunized mice, wherein the hybridoma secretes a monoclonal antibody that binds PAR-2, also are provided herein.

Although human, partially human, or humanized antibodies will be suitable for many applications, particularly those involving administration of the antibody to a human subject, other types of antigen binding proteins will be suitable for certain applications. The non-human antibodies of the invention can be, for example, derived from any antibody-producing animal, such as mouse, rat, rabbit, goat, donkey, or non-human primate (such as monkey (e.g., cynomolgous or rhesus monkey) or ape (e.g., chimpanzee)). Non-human antibodies of the invention can be used, for example, in in vitro and cell-culture based applications, or any other application where an immune response to the antibody of the invention does not occur, is insignificant, can be prevented, is not a concern, or is desired. In one embodiment, a non-human antibody of the invention is administered to a non-human subject. In another embodiment, the non-human antibody does not elicit an immune response in the non-human subject. In another embodiment, the non-human antibody is from the same species as the non-human subject, e.g., a mouse antibody of the invention is administered to a mouse. An antibody from a particular species can be made by, for example, immunizing an animal of that species with the desired immunogen (e.g., a soluble PAR-2 polypeptide) or using an artificial system for generating antibodies of that species (e.g., a bacterial or phage display-based system for generating antibodies of a particular species), or by converting an antibody from one species into an antibody from another species by replacing, e.g., the constant region of the antibody with a constant region from the other species, or by replacing one or more amino acid residues of the antibody so that it more closely resembles the sequence of an antibody from the other species. In one embodiment, the antibody is a chimeric antibody comprising amino acid sequences derived from antibodies from two or more different species.

Antigen binding proteins may be prepared by any of a number of conventional techniques. For example, they may be purified from cells that naturally express them (e.g., an antibody can be purified from a hybridoma that produces it), or produced in recombinant expression systems, using any technique known in the art. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York

(1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988).

Any expression system known in the art can be used to make the recombinant polypeptides of the invention. In general, host cells are transformed with a recombinant expression vector that comprises DNA encoding a desired polypeptide. Among the host cells that may be employed are prokaryotes, yeast or higher eukaryotic cells. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Higher eukaryotic cells include insect cells and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., 1981, Cell 23:175), L cells, 293 cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, BHK (ATCC CRL 10) cell lines, and the CVI/EBNA cell line derived from the African green monkey kidney cell line CVI (ATCC CCL 70) as described by McMahan et al., 1991, EMBO J. 10: 2821. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985).

The transformed cells can be cultured under conditions that promote expression of the polypeptide, and the polypeptide recovered by conventional protein purification procedures. One such purification procedure includes the use of affinity chromatography, e.g., over a matrix having all or a portion (e.g., the extracellular domain) of PAR-2 bound thereto. Polypeptides contemplated for use herein include substantially homogeneous recombinant mammalian anti-PAR-2 antibody polypeptides substantially free of contaminating endogenous materials.

Antigen binding proteins may be prepared, and screened for desired properties, by any of a number of known techniques. Certain of the techniques involve isolating a nucleic acid encoding a polypeptide chain (or portion thereof) of an antigen binding protein of interest (e.g., an anti-PAR-2 antibody), and manipulating the nucleic acid through recombinant DNA technology. The nucleic acid may be fused to another nucleic acid of interest, or altered (e.g., by mutagenesis or other conventional techniques) to add, delete, or substitute one or more amino acid residues, for example.

In one aspect, the present invention provides antigen-binding fragments of an anti-PAR-2 antibody of the invention. Such fragments can consist entirely of antibody-derived sequences or can comprise additional sequences. Examples of antigen-binding fragments include Fab, F(ab')₂, single chain antibodies, diabodies, triabodies, tetrabodies, and domain antibodies. Other examples are provided in Lunde et al., 2002, Biochem. Soc. Trans. 30:500-06.

Single chain antibodies may be formed by linking heavy and light chain variable domain (Fv region) fragments via an amino acid bridge (short peptide linker), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable domain polypeptides (V_L and V_H). The resulting polypeptides can fold back on themselves to form antigen-binding monomers, or they can form multimers (e.g., dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt et al., 1997, Prot. Eng. 10:423; Kortt et al., 2001, Biomol. Eng. 18:95-108). By combining different V_L and V_H-comprising polypeptides, one can form multimeric scFvs that bind to different epitopes (Kriangkum et al., 2001, Biomol. Eng. 18:31-40). Techniques developed for the production of single chain antibodies include those described in

U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879; Ward et al., 1989, Nature 334:544, de Graaf et al., 2002, Methods Mol Biol. 178:379-87.

Antigen binding proteins (e.g., antibodies, antibody fragments, and antibody derivatives) of the invention can comprise any constant region known in the art. The light chain constant region can be, for example, a kappa- or lambda-type light chain constant region, e.g., a human kappa- or lambda-type light chain constant region. The heavy chain constant region can be, for example, an alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant regions, e.g., a human alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region. In one embodiment, the light or heavy chain constant region is a fragment, derivative, variant, or mutein of a naturally occurring constant region.

Techniques are known for deriving an antibody of a different subclass or isotype from an antibody of interest, i.e., subclass switching. Thus, IgG antibodies may be derived from an IgM antibody, for example, and vice versa. Such techniques allow the preparation of new antibodies that possess the antigen-binding properties of a given antibody (the parent antibody), but also exhibit biological properties associated with an antibody isotype or subclass different from that of the parent antibody. Recombinant DNA techniques may be employed. Cloned DNA encoding particular antibody polypeptides may be employed in such procedures, e.g., DNA encoding the constant domain of an antibody of the desired isotype. See also Lantto et al., 2002, Methods Mol. Biol. 178:303-16. Moreover, if an IgG4 is desired, it may also be desired to introduce a point mutation (CPSCP->CPPCP) in the hinge region as described in Bloom et al., 1997, Protein Science 6:407, incorporated by reference herein) to alleviate a tendency to form intra-H chain disulfide bonds that can lead to heterogeneity in the IgG4 antibodies.

Moreover, techniques for deriving antigen binding proteins having different properties (i.e., varying affinities for the antigen to which they bind) are also known. One such technique, referred to as chain shuffling, involves displaying immunoglobulin variable domain gene repertoires on the surface of filamentous bacteriophage, often referred to as phage display. Chain shuffling has been used to prepare high affinity antibodies to the hapten 2-phenyloxazol-5-one, as described by Marks et al., 1992, BioTechnology, 10:779.

In particular embodiments, antigen binding proteins of the present invention have a binding affinity (K_a) for PAR-2 of at least 10^6 . In other embodiments, the antigen binding proteins exhibit a K_a of at least 10^7 , at least 10^8 , at least 10^9 , or at least 10^{10} . In another embodiment, the antigen binding protein exhibits a K_a substantially the same as that of an antibody described herein in the Examples.

In another embodiment, the present invention provides an antigen binding protein that has a low dissociation rate from PAR-2. In one embodiment, the antigen binding protein has a K_{off} of $1 \times 10^{-4} \text{ s}^{-1}$ or lower. In another embodiment, the K_{off} is $5 \times 10^{-5} \text{ s}^{-1}$ or lower. In another embodiment, the K_{off} is substantially the same as an antibody described herein in the Examples. In another embodiment, the antigen binding protein binds to PAR-2 with substantially the same K_{off} as an antibody described herein in the Examples.

In another aspect, the present invention provides an antigen binding protein that inhibits an activity of PAR-2, for example Ca^{2+} mobilization. In one embodiment, the antigen binding protein has an IC_{50} of 1000 nM or lower. In another embodiment, the IC_{50} is 100 nM or lower; in another embodiment, the IC_{50} is 10 nM or lower. In another embodiment, the IC_{50} is substantially the same as that of an antibody described

herein in the Examples. In another embodiment, the antigen binding protein inhibits an activity of PAR-2 with substantially the same IC_{50} as an antibody described herein in the Examples.

In another embodiment, the present invention provides an antigen binding protein that binds to full-length PAR-2 and binds to a lesser extent to cleaved PAR-2. In various embodiments, the antigen binding protein binds to full-length PAR-2 by at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, and 99.9% less than it binds to cleaved PAR-2.

In another aspect, the present invention provides an antigen binding protein that binds at or near the protease cleavage site of human PAR-2. Antigen binding proteins that bind to the protease cleavage site can be made using any technique known in the art. For example, such antigen binding proteins can be isolated using the full-length PAR-2 polypeptide (e.g., in a membrane-bound preparation), a soluble extracellular domain fragment of PAR-2, or a smaller fragment of the PAR-2 extracellular domain comprising or consisting of the protease cleavage site (examples of which are provided herein). Antigen binding proteins so isolated can be screened to determine their binding specificity using any method known in the art (examples of which are provided herein).

In another embodiment, the present invention provides an antigen binding protein that competes for binding to PAR-2 with an antibody disclosed herein. Such competitive ability can be determined by methods that are well-known in the art, for example by competition in binding to PAR-2/Fc in a Western blot (or another peptide-based assay), or by competition in a Ca^{2+} flux assay as described herein. In one aspect, an antigen binding protein that competes for binding to PAR-2 with an antibody disclosed herein binds the same epitope as the antibody. In another aspect, the antigen binding protein that competes for binding to PAR-2 with an antibody disclosed herein inhibits proteolytic activation of PAR-2.

In another aspect, the present invention provides an antigen binding protein that binds to human PAR-2 expressed on the surface of a cell and, when so bound, inhibits PAR-2 signaling activity in the cell without causing a significant reduction in the amount of PAR-2 on the surface of the cell. Any method for determining or estimating the amount of PAR-2 on the surface and/or in the interior of the cell can be used. In one embodiment, the present invention provides an antigen binding protein that binds to or near the protease cleavage site of a human PAR-2 expressed on the surface of a cell and, when so bound, inhibits PAR-2 signaling activity in the cell without significantly increasing the rate of internalization of the PAR-2 from the surface of the cell. In other embodiments, binding of the antigen binding protein to the PAR-2-expressing cell causes less than about 75%, 50%, 40%, 30%, 20%, 15%, 10%, 5%, 1%, or 0.1% of the cell-surface PAR-2 to be internalized.

In another aspect, the present invention provides an antigen binding protein having a half-life of at least one day in vitro or in vivo (e.g., when administered to a human subject). In one embodiment, the antigen binding protein has a half-life of at least three days. In another embodiment, the antigen binding protein has a half-life of four days or longer. In another embodiment, the antigen binding protein has a half-life of eight days or longer. In another embodiment, the antigen binding protein is derivatized or modified such that it has a longer half-life as compared to the underivatized or unmodified antigen binding protein. In another embodiment, the antigen binding protein contains one or more point mutations

to increase serum half life, such as described in WO 00/09560, published Feb. 24, 2000, incorporated by reference.

The present invention further provides multi-specific antigen binding proteins, for example, bispecific antigen binding protein, e.g., antigen binding protein that bind to two different epitopes of PAR-2, or to an epitope of PAR-2 and an epitope of another molecule, via two different antigen binding sites or regions. Moreover, bispecific antigen binding protein as disclosed herein can comprise a PAR-2 binding site from one of the herein-described antibodies and a second PAR-2 binding region from another of the herein-described antibodies, including those described herein by reference to other publications. Alternatively, a bispecific antigen binding protein may comprise an antigen binding site from one of the herein described antibodies and a second antigen binding site from another PAR-2 antibody that is known in the art, or from an antibody that is prepared by known methods or the methods described herein.

Numerous methods of preparing bispecific antibodies are known in the art, and discussed in U.S. patent application Ser. No. 09/839,632, filed Apr. 20, 2001 (incorporated by reference herein). Such methods include the use of hybrid-hybridomas as described by Milstein et al., 1983, *Nature* 305:537, and others (U.S. Pat. No. 4,474,893, U.S. Pat. No. 6,106,833), and chemical coupling of antibody fragments (Brennan et al., 1985, *Science* 229:81; Glennie et al., 1987, *J. Immunol* 139:2367; U.S. Pat. No. 6,010,902). Moreover, bispecific antibodies can be produced via recombinant means, for example by using leucine zipper moieties (i.e., from the Fos and Jun proteins, which preferentially form heterodimers; Kostelny et al., 1992, *J. Immunol* 148:1547) or other lock and key interactive domain structures as described in U.S. Pat. No. 5,582,996. Additional useful techniques include those described in Kortt et al., 1997, *supra*; U.S. Pat. No. 5,959,083; and U.S. Pat. No. 5,807,706.

In another aspect, the antigen binding protein of the present invention comprises a derivative of an antibody. The derivatized antibody can comprise any molecule or substance that imparts a desired property to the antibody, such as increased half-life in a particular use. The derivatized antibody can comprise, for example, a detectable (or labeling) moiety (e.g., a radioactive, colorimetric, antigenic or enzymatic molecule, a detectable bead (such as a magnetic or electrodense (e.g., gold) bead), or a molecule that binds to another molecule (e.g., biotin or streptavidin)), a therapeutic or diagnostic moiety (e.g., a radioactive, cytotoxic, or pharmaceutically active moiety), or a molecule that increases the suitability of the antibody for a particular use (e.g., administration to a subject, such as a human subject, or other in vivo or in vitro uses). Examples of molecules that can be used to derivatize an antibody include albumin (e.g., human serum albumin) and polyethylene glycol (PEG). Albumin-linked and PEGylated derivatives of antibodies can be prepared using techniques well known in the art. In one embodiment, the antibody is conjugated or otherwise linked to transthyretin (TTR) or a TTR variant. The TTR or TTR variant can be chemically modified with, for example, a chemical selected from the group consisting of dextran, poly(n-vinyl pyrrolidone), polyethylene glycols, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols and polyvinyl alcohols. US Pat. App. No. 20030195154.

In another aspect, the present invention provides methods of screening for a molecule that binds to PAR-2 using the antigen binding proteins of the present invention. Any suitable screening technique can be used. In one embodiment, a

PAR-2 molecule, or a fragment thereof to which an antigen binding protein of the present invention binds, is contacted with the antigen binding protein of the invention and with another molecule, wherein the other molecule binds to PAR-2 if it reduces the binding of the antigen binding protein to PAR-2. Binding of the antigen binding protein can be detected using any suitable method, e.g., an ELISA. Detection of binding of the antigen binding protein to PAR-2 can be simplified by detectably labeling the antigen binding protein, as discussed above. In another embodiment, the PAR-2-binding molecule is further analyzed to determine whether it inhibits PAR-2 activation and/or signaling.

Nucleic Acids

In one aspect, the present invention provides isolated nucleic acid molecules. The nucleic acids comprise, for example, polynucleotides that encode all or part of an antigen binding protein, for example, one or both chains of an antibody of the invention, or a fragment, derivative, mutein, or variant thereof, polynucleotides sufficient for use as hybridization probes, PCR primers or sequencing primers for identifying, analyzing, mutating or amplifying a polynucleotide encoding a polypeptide, anti-sense nucleic acids for inhibiting expression of a polynucleotide, and complementary sequences of the foregoing. The nucleic acids can be any length. They can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 750, 1,000, 1,500, 3,000, 5,000 or more nucleotides in length, and/or can comprise one or more additional sequences, for example, regulatory sequences, and/or be part of a larger nucleic acid, for example, a vector. The nucleic acids can be single-stranded or double-stranded and can comprise RNA and/or DNA nucleotides, and artificial variants thereof (e.g., peptide nucleic acids).

Nucleic acids encoding antibody polypeptides (e.g., heavy or light chain, variable domain only, or full length) may be isolated from B-cells of mice that have been immunized with PAR-2. The nucleic acid may be isolated by conventional procedures such as polymerase chain reaction (PCR).

The invention further provides nucleic acids that hybridize to other nucleic acids under particular hybridization conditions. Methods for hybridizing nucleic acids are well-known in the art. See, e.g., *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. As defined herein, a moderately stringent hybridization condition uses a prewashing solution containing 5× sodium chloride/sodium citrate (SSC), 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6×SSC, and a hybridization temperature of 55° C. (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of 42° C.), and washing conditions of 60° C., in 0.5×SSC, 0.1% SDS. A stringent hybridization condition hybridizes in 6×SSC at 45° C., followed by one or more washes in 0.1×SSC, 0.2% SDS at 68° C. Furthermore, one of skill in the art can manipulate the hybridization and/or washing conditions to increase or decrease the stringency of hybridization such that nucleic acids comprising nucleotide sequences that are at least 65, 70, 75, 80, 85, 90, 95, 98 or 99% identical to each other typically remain hybridized to each other. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by, for example, Sambrook, Fritsch, and Maniatis (1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11; and *Current Protocols in Molecular Biology*, 1995, Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4), and can be readily

determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA.

Changes can be introduced by mutation into a nucleic acid, thereby leading to changes in the amino acid sequence of a polypeptide (e.g., an antigen binding protein) that it encodes. Mutations can be introduced using any technique known in the art. In one embodiment, one or more particular amino acid residues are changed using, for example, a site-directed mutagenesis protocol. In another embodiment, one or more randomly selected residues is changed using, for example, a random mutagenesis protocol. However it is made, a mutant polypeptide can be expressed and screened for a desired property (e.g., binding to PAR-2 or blocking the proteolytic activation of PAR-2).

Mutations can be introduced into a nucleic acid without significantly altering the biological activity of a polypeptide that it encodes. For example, one can make nucleotide substitutions leading to amino acid substitutions at non-essential amino acid residues. In one embodiment, a nucleotide sequence, or a desired fragment, variant, or derivative thereof, is mutated such that it encodes an amino acid sequence comprising one or more deletions or substitutions of amino acid residues. In another embodiment, the mutagenesis inserts an amino acid adjacent to one or more amino acid residues. Alternatively, one or more mutations can be introduced into a nucleic acid that selectively change the biological activity (e.g., binding of PAR-2, inhibiting proteolytic activation of PAR-2, etc.) of a polypeptide that it encodes. For example, the mutation can quantitatively or qualitatively change the biological activity. Examples of quantitative changes include increasing, reducing or eliminating the activity. Examples of qualitative changes include changing the antigen specificity of an antigen binding protein.

In another aspect, the present invention provides nucleic acid molecules that are suitable for use as primers or hybridization probes for the detection of nucleic acid sequences of the invention. A nucleic acid molecule of the invention can comprise only a portion of a nucleic acid sequence encoding a full-length polypeptide of the invention, for example, a fragment that can be used as a probe or primer or a fragment encoding an active portion (e.g., a PAR-2 binding portion) of a polypeptide of the invention.

Probes based on the sequence of a nucleic acid of the invention can be used to detect the nucleic acid or similar nucleic acids, for example, transcripts encoding a polypeptide of the invention. The probe can comprise a label group, e.g., a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used to identify a cell that expresses the polypeptide.

In another aspect, the present invention provides vectors comprising a nucleic acid encoding a polypeptide of the invention or a portion thereof. Examples of vectors include, but are not limited to, plasmids, viral vectors, non-episomal mammalian vectors and expression vectors, for example, recombinant expression vectors.

The recombinant expression vectors of the invention can comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell. The recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells (e.g., SV40 early gene enhancer, Rous sarcoma virus promoter and cytomegalovirus promoter), those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences,

see Voss et al., 1986, Trends Biochem. Sci. 11:287, Maniatis et al., 1987, Science 236:1237, incorporated by reference herein in their entireties), and those that direct inducible expression of a nucleotide sequence in response to particular treatment or condition (e.g., the metallothionin promoter in mammalian cells and the tet-responsive and/or streptomycin responsive promoter in both prokaryotic and eukaryotic systems (see id.)). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein.

In another aspect, the present invention provides host cells into which a recombinant expression vector of the invention has been introduced. A host cell can be any prokaryotic cell (for example, *E. coli*) or eukaryotic cell (for example, yeast, insect, or mammalian cells (e.g., CHO cells)). Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die), among other methods.

Indications

In one aspect, the present invention provides methods of treating a subject. The method can, for example, have a generally salubrious effect on the subject, e.g., it can increase the subject's expected longevity. Alternatively, the method can, for example, treat, prevent, cure, relieve, or ameliorate ("treat") a disease, disorder, condition, or illness ("a condition"). Among the conditions to be treated in accordance with the present invention are conditions characterized by inappropriate expression or activity of PAR-2. In some such conditions, the expression or activity level is too high, and the treatment comprises administering a PAR-2 antagonist as described herein.

Specific medical conditions and diseases that are treatable or preventable with the antigen binding proteins of this invention include inflammatory conditions of the gastrointestinal system, including coeliac disease, Crohn's disease; ulcerative colitis; idiopathic gastroparesis; pancreatitis, including chronic pancreatitis; inflammatory bowel disease and ulcers, including gastric and duodenal ulcers. The antigen binding proteins of this invention are also useful in treating or ameliorating inflammatory conditions of the airway, such as asthma, chronic obstructive pulmonary disease, and the like.

Rheumatic disorders that are treatable with the antigen binding proteins of this invention include adult and juvenile rheumatoid arthritis; scleroderma; systemic lupus erythematosus; gout; osteoarthritis; polymyalgia rheumatica; seronegative spondylarthropathies, including ankylosing spondylitis, and Reiter's disease, psoriatic arthritis and chronic Lyme arthritis. Also treatable or preventable with these polypeptides are Still's disease and uveitis associated with rheumatoid arthritis. In addition, the polypeptide therapies of the

invention are used in treating disorders resulting in inflammation of the voluntary muscle and other muscles, including dermatomyositis, inclusion body myositis, polymyositis, and lymphangiomyomatosis.

The disorders described herein can be treated with the antigen binding proteins of this invention in combination with other cytokines, cytokine inhibitors and reagents (also referred to herein as immunomodulators). For example, immunomodulators include IL-18 antagonists such as soluble IL-18 receptor, antibodies to IL-18 or the IL-18 receptor, IL-18 binding protein; TNF inhibitors, including ENBREL®; IL-1 inhibitors, including soluble forms of type I IL-1R, type II IL-1R, antibodies to IL-1, antibodies to type I IL-1R; and or other active agents that are effective in treating the disclosed medical conditions and diseases.

The compositions and/or methods of the present invention also can be used, for example, in cosmetic treatments, in veterinary treatments, to increase longevity, to treat reproductive defects, and to treat a variety of PAR-2 related disorders. In addition, in certain such conditions, the expression or activity level of PAR-2 is too low, and the treatment comprises administering a PAR-2 agonist; such treatments are also comprehended herein.

Therapeutic Methods and Administration of Antigen Binding Proteins

Certain methods provided herein comprise administering a PAR-2 binding antigen binding protein to a subject, thereby reducing a PAR-2-induced biological response that plays a role in a particular condition. In particular embodiments, methods of the invention involve contacting endogenous PAR-2 with a PAR-2 binding antigen binding protein, e.g., via administration to a subject or in an ex vivo procedure.

The term "treatment" encompasses alleviation or prevention of at least one symptom or other aspect of a disorder, or reduction of disease severity, and the like. An antigen binding protein need not effect a complete cure, or eradicate every symptom or manifestation of a disease, to constitute a viable therapeutic agent. As is recognized in the pertinent field, drugs employed as therapeutic agents may reduce the severity of a given disease state, but need not abolish every manifestation of the disease to be regarded as useful therapeutic agents. Similarly, a prophylactically administered treatment need not be completely effective in preventing the onset of a condition in order to constitute a viable prophylactic agent. Simply reducing the impact of a disease (for example, by reducing the number or severity of its symptoms, or by increasing the effectiveness of another treatment, or by producing another beneficial effect), or reducing the likelihood that the disease will occur or worsen in a subject, is sufficient. One embodiment of the invention is directed to a method comprising administering to a patient a PAR-2 antagonist in an amount and for a time sufficient to induce a sustained improvement over baseline of an indicator that reflects the severity of the particular disorder.

As is understood in the pertinent field, pharmaceutical compositions comprising the molecules of the invention are administered to a subject in a manner appropriate to the indication. Pharmaceutical compositions may be administered by any suitable technique, including but not limited to parenterally, topically, or by inhalation. If injected, the pharmaceutical composition can be administered, for example, via intra-articular, intravenous, intramuscular, intralesional, intraperitoneal or subcutaneous routes, by bolus injection, or continuous infusion. Localized administration, e.g. at a site of disease or injury is contemplated, as are transdermal delivery and sustained release from implants. Delivery by inhalation includes, for example, nasal or oral inhalation, use of a nebu-

lizer, inhalation of the antagonist in aerosol form, and the like. Other alternatives include eyedrops; oral preparations including pills, syrups, lozenges or chewing gum; and topical preparations such as lotions, gels, sprays, and ointments.

Use of antigen binding proteins in ex vivo procedures also is contemplated. For example, a patient's blood or other bodily fluid may be contacted with an antigen binding protein that binds PAR-2 ex vivo. The antigen binding protein may be bound to a suitable insoluble matrix or solid support material.

Advantageously, antigen binding proteins are administered in the form of a composition comprising one or more additional components such as a physiologically acceptable carrier, excipient or diluent. Optionally, the composition additionally comprises one or more physiologically active agents, for example, a second inflammation- or immune-inhibiting substance, an anti-angiogenic substance, an analgesic substance, etc., non-exclusive examples of which are provided herein. In various particular embodiments, the composition comprises one, two, three, four, five, or six physiologically active agents in addition to a PAR-2 binding antigen binding protein

In one embodiment, the pharmaceutical composition comprise an antigen binding protein of the invention together with one or more substances selected from the group consisting of a buffer, an antioxidant such as ascorbic acid, a low molecular weight polypeptide (such as those having fewer than 10 amino acids), a protein, an amino acid, a carbohydrate such as glucose, sucrose or dextrans, a chelating agent such as EDTA, glutathione, a stabilizer, and an excipient. Neutral buffered saline or saline mixed with conspecific serum albumin are examples of appropriate diluents. In accordance with appropriate industry standards, preservatives such as benzyl alcohol may also be added. The composition may be formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Suitable components are nontoxic to recipients at the dosages and concentrations employed. Further examples of components that may be employed in pharmaceutical formulations are presented in Remington's Pharmaceutical Sciences, 16th Ed. (1980) and 20th Ed. (2000), Mack Publishing Company, Easton, Pa.

Kits for use by medical practitioners include a PAR-2-inhibiting substance of the invention and a label or other instructions for use in treating any of the conditions discussed herein. In one embodiment, the kit includes a sterile preparation of one or more PAR-2 binding antigen binding proteins, which may be in the form of a composition as disclosed above, and may be in one or more vials.

Dosages and the frequency of administration may vary according to such factors as the route of administration, the particular antigen binding proteins employed, the nature and severity of the disease to be treated, whether the condition is acute or chronic, and the size and general condition of the subject. Appropriate dosages can be determined by procedures known in the pertinent art, e.g. in clinical trials that may involve dose escalation studies.

A PAR-2 inhibiting substance of the invention may be administered, for example, once or more than once, e.g., at regular intervals over a period of time. In particular embodiments, an antigen binding protein is administered over a period of at least a month or more, e.g., for one, two, or three months or even indefinitely. For treating chronic conditions, long-term treatment is generally most effective. However, for treating acute conditions, administration for shorter periods, e.g. from one to six weeks, may be sufficient. In general, the antigen binding protein is administered until the patient manifests a medically relevant degree of improvement over baseline for the chosen indicator or indicators.

Particular embodiments of the present invention involve administering an antigen binding protein at a dosage of from about 1 ng of antigen binding protein per kg of subject's weight per day ("1 ng/kg/day") to about 10 mg/kg/day, more preferably from about 500 ng/kg/day to about 5 mg/kg/day, and most preferably from about 5 µg/kg/day to about 2 mg/kg/day, to a subject. In additional embodiments, an antigen binding protein is administered to adults one time per week, two times per week, or three or more times per week, to treat a PAR-2 mediated disease, condition or disorder, e.g., a medical disorder disclosed herein. If injected, the effective amount of antigen binding protein per adult dose may range from 1-20 mg/m², and preferably is about 5-12 mg/m². Alternatively, a flat dose may be administered; the amount may range from 5-100 mg/dose. One range for a flat dose is about 20-30 mg per dose. In one embodiment of the invention, a flat dose of 25 mg/dose is repeatedly administered by injection. If a route of administration other than injection is used, the dose is appropriately adjusted in accordance with standard medical practices. One example of a therapeutic regimen involves injecting a dose of about 20-30 mg of antigen binding protein to one to three times per week over a period of at least three weeks, though treatment for longer periods may be necessary to induce the desired degree of improvement. For pediatric subjects (age 4-17), one exemplary suitable regimen involves the subcutaneous injection of 0.4 mg/kg, up to a maximum dose of 25 mg of antigen binding protein administered two or three times per week.

Particular embodiments of the methods provided herein involve subcutaneous injection of from 0.5 mg to 10 mg, preferably from 3 to 5 mg, of an antigen binding protein, once or twice per week. Another embodiment is directed to pulmonary administration (e.g., by nebulizer) of 3 or more mg of antigen binding protein once a week.

Examples of therapeutic regimens provided herein comprise subcutaneous injection of an antigen binding protein once a week, at a dose of 1.5 to 3 mg, to treat a condition in which PAR-2 signaling plays a role. Examples of such conditions are provided herein and include, for example, rheumatic conditions as previously described, and other conditions in which excessive inflammation plays a role (described herein; for example, inflammatory bowel disease, pancreatitis, etc). Weekly administration of antigen binding protein is continued until a desired result is achieved, e.g., the subject's symptoms subside. Treatment may resume as needed, or, alternatively, maintenance doses may be administered.

Other examples of therapeutic regimens provided herein comprise subcutaneous or intravenous administration of a dose of 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 15, or 20 milligrams of a PAR-2 inhibitor of the present invention per kilogram body mass of the subject (mg/kg). The dose can be administered once to the subject, or more than once at a certain interval, for example, once a day, three times a week, twice a week, once a week, three times a month, twice a month, once a month, once every two months, once every three months, once every six months, or once a year. The duration of the treatment, and any changes to the dose and/or frequency of treatment, can be altered or varied during the course of treatment in order to meet the particular needs of the subject.

In another embodiment, an antigen binding protein is administered to the subject in an amount and for a time sufficient to induce an improvement, preferably a sustained improvement, in at least one indicator that reflects the severity of the disorder that is being treated. Various indicators that reflect the extent of the subject's illness, disease or condition may be assessed for determining whether the amount and time of the treatment is sufficient. Such indicators include, for

example, clinically recognized indicators of disease severity, symptoms, or manifestations of the disorder in question. In one embodiment, an improvement is considered to be sustained if the subject exhibits the improvement on at least two occasions separated by two to four weeks. The degree of improvement generally is determined by a physician, who may make this determination based on signs, symptoms, biopsies, or other test results, and who may also employ questionnaires that are administered to the subject, such as quality-of-life questionnaires developed for a given disease.

Elevated levels of PAR-2 and/or activation of PAR-2 are associated with a number of disorders, including, for example, inflammatory conditions of the skin, joints, gastrointestinal system and/or airway. Subjects with a given disorder may be screened, to identify those individuals who have elevated PAR-2 activation, thereby identifying the subjects who may benefit most from treatment with a PAR-2 binding antigen binding protein. Thus, treatment methods provided herein optionally comprise a first step of measuring a subject's PAR-2 activation levels. An antigen binding protein may be administered to a subject in whom PAR-2 activation is elevated above normal.

A subject's levels of PAR-2 activity may be monitored before, during and/or after treatment with an antigen binding protein, to detect changes, if any, in PAR-2 activity. For some disorders, the incidence of elevated PAR-2 activity may vary according to such factors as the stage of the disease or the particular form of the disease. Known techniques may be employed for measuring PAR-2 activity, e.g., in a subject's serum, blood or tissue samples. PAR-2 activity may be measured using any suitable technique.

Particular embodiments of methods and compositions of the invention involve the use of an antigen binding protein and one or more additional PAR-2 antagonists, for example, two or more antigen binding proteins of the invention, or an antigen binding protein of the invention and one or more other PAR-2 antagonists. In further embodiments, antigen binding protein is administered alone or in combination with other agents useful for treating the condition with which the patient is afflicted. Examples of such agents include both proteinaceous and non-proteinaceous drugs. When multiple therapeutics are co-administered, dosages may be adjusted accordingly, as is recognized in the pertinent art. "Co-administration" and combination therapy are not limited to simultaneous administration, but also include treatment regimens in which an antigen binding protein is administered at least once during a course of treatment that involves administering at least one other therapeutic agent to the patient.

Examples of other agents that may be co-administered with an antigen binding protein are other antigen binding proteins or therapeutic polypeptides that are chosen according to the particular condition to be treated. Alternatively, non-proteinaceous drugs that are useful in treating one of the particular conditions discussed above may be co-administered with a PAR-2 antagonist.

Combination Therapy

In another aspect, the present invention provides a method of treating a subject with a PAR-2 inhibiting antigen binding protein and one or more other treatments. In one embodiment, such a combination therapy achieves synergy or an additive effect by, for example, attacking multiple sites or molecular targets in a tumor. Types of combination therapies that can be used in connection with the present invention include inhibiting or activating (as appropriate) multiple nodes in a single disease-related pathway, multiple pathways in a target cell, and multiple cell types within a target tissue.

In another embodiment, a combination therapy method comprises administering to the subject two, three, four, five, six, or more of the PAR-2 agonists or antagonists described herein. In another embodiment, the method comprises administering to the subject two or more treatments that together inhibit or activate (directly or indirectly) PAR-2-mediated signal transduction. Examples of such methods include using combinations of two or more PAR-2 inhibiting antigen binding proteins, of a PAR-2 inhibiting antigen binding protein and one or more other therapeutic moiety having anti-inflammatory properties (for example, non-steroidal anti-inflammatory agents, steroids, and/or immunomodulators), or of a PAR-2 inhibiting antigen binding protein and one or more other treatments (e.g., surgery, ultrasound, or treatment effective to reduce inflammation). Furthermore, one or more anti-PAR-2 antibodies or antibody derivatives can be used in combination with one or more molecules or other treatments, wherein the other molecule(s) and/or treatment(s) do not directly bind to or affect PAR-2, but which combination is effective for treating or preventing the condition being treated. In one embodiment, one or more of the molecule(s) and/or treatment(s) treats or prevents a condition that is caused by one or more of the other molecule(s) or treatment(s) in the course of therapy, e.g., nausea, fatigue, alopecia, cachexia, insomnia, etc. In every case where a combination of molecules and/or other treatments is used, the individual molecule(s) and/or treatment(s) can be administered in any order, over any length of time, which is effective, e.g., simultaneously, consecutively, or alternately. In one embodiment, the method of treatment comprises completing a first course of treatment with one molecule or other treatment before beginning a second course of treatment. The length of time between the end of the first course of treatment and beginning of the second course of treatment can be any length of time that allows the total course of therapy to be effective, e.g., seconds, minutes, hours, days, weeks, months, or even years.

In another embodiment, the method comprises administering one or more of the PAR-2 antagonists described herein and one or more other treatments (e.g., a therapeutic or palliative treatment). Where a method comprises administering more than one treatment to a subject, it is to be understood that the order, timing, number, concentration, and volume of the administrations is limited only by the medical requirements and limitations of the treatment, i.e., two treatments can be administered to the subject, e.g., simultaneously, consecutively, alternately, or according to any other regimen.

The following examples, both actual and prophetic, are provided for the purpose of illustrating specific embodiments or features of the instant invention and do not limit its scope.

Example 1

Preparation of Monoclonal Antibodies

PAR-2 polypeptides may be employed as immunogens in generating monoclonal antibodies by conventional techniques, e.g., techniques described in U.S. Pat. No. 5,599,905, hereby incorporated by reference. Additional techniques are known in the art, for example, the RIMMS (Repetitive Immunizations Multiple Sites) strategy (Kilpatrick et al., *Hybridoma* 16(4):381-9; 1997.) It is recognized that polypeptides in various forms may be employed as immunogens, e.g., full length proteins, fragments thereof, fusion proteins such as Fc fusions, cells expressing the recombinant protein on the cell surface, etc.

To summarize an example of such a procedure, a loop 1 peptide of PAR-2 (TNRSSKGRSLIGKVDGTS; amino acids 29 through 46 of SEQ ID NO:2), having an additional C-terminal cysteine residue to facilitate conjugation, is conjugated to maleimide-activated keyhole limpet hemocyanin (KLH; obtainable for example from Pierce Biotechnology Inc., Rockford, Ill.) to yield a PAR-2 immunogen. For a first immunization, 100 micrograms of immunogen (containing 50 micrograms of peptide) is emulsified in complete Freund's adjuvant (CFA) at 1:1 ratio by volume and injected subcutaneously in a final volume of 200 microliters for each mouse.

Immunized animals are boosted three to four more times with additional immunogen to increase the antigen-specific response, at intervals of two to four weeks (although longer intervals may be employed. For example, a second injection of 50 micrograms of immunogen (containing 25 micrograms of peptide) mixed with incomplete Freund's adjuvant in a final volume of 200 ul is injected subcutaneously into each mouse about four weeks days after the primary immunization. A third injection (20 micrograms of immunogen containing 10 micrograms of peptide mixed with an adjuvant such as Ribi adjuvant) may be given by subcutaneous and/or intraperitoneal route from about 14 to about 28 days after the second injection. If desired, a fourth injection (20 micrograms of immunogen containing 10 micrograms of peptide mixed with incomplete Freund's adjuvant) may be given by subcutaneous and/or intraperitoneal route from about 14 to about 28 days after the third injection. A final injection is given, usually about five days prior to fusion, utilizing 50 micrograms of immunogen containing 25 micrograms of peptide in PBS, by intraperitoneal injection.

Serum samples may be periodically taken by retro-orbital bleeding or tail-tip excision for testing by peptide ELISA (enzyme-linked immunosorbent assay), or another suitable assay, to evaluate antibody titer. At the time of fusion, the animals are sacrificed, splenocytes harvested, and fused to the murine myeloma cell line SP2/O (ATCC CRL 1581) or another suitable cell line, several of which are known in the art. The resulting hybridoma cell lines are plated in multiple microtiter plates in a HAT selective medium (hypoxanthine, aminopterin, and thymidine) to facilitate proliferation of spleen cell-myeloma hybrid cells.

Hybridoma clones thus generated are screened for reactivity with PAR-2. Initial screening of hybridoma supernatants may utilize a peptide ELISA, a whole cell ELISA and/or a cell-based assay suitable for high-throughput screening (fluorometric microvolume assay technology or FMAT, substantially as described by Fiscella, et al., *Nature Biotechnology* 21:302-307; 2003). Hybridomas that are positive in this screening method may be further cultured to provide larger amounts of antibody, which can then be purified as described below and screened by additional cell-based assay(s) (for example, a flash plate assay using cells co-expressing apoaequorin, a Ca²⁺-sensitive photoprotein, substantially as described by Le Poul et al., *J. Biomol. Screen.* 7(1):57-65; 2002, and PAR-2), or a fluorometric imaging plate reader (FLIPR) assay, which is used to determine changes in intracellular Ca²⁺ levels, substantially as described in S. Pitchford, *Genetic Engineering News* vol. 18, Number 15 (1998) and/or Sullivan et al., *Methods in Molecular Biology* vol. 114, pp 125-133 (1999), which are incorporated by reference herein.

Selected hybridomas can be further cloned and tested to ensure stable production of monoclonal antibody. Hybridomas can be cultured in vitro, or passaged as ascites fluid in suitable host mammals. The resulting monoclonal antibodies may be purified by ammonium sulfate precipitation followed

31

by gel exclusion chromatography, and/or affinity chromatography based on binding of antibody to Protein G, for example. Several hybridomas were generated and tested for binding in a whole-cell ELISA using PAR-2 expressing cells, and in a flash plate assay; results are shown in Table 1 below.

TABLE 1

Clone ID	Binding	Blocking in FLASH
#47	+	+
#21	+	N.A.
#48	+	+
#6	+	+
#15	+	N.A.
#13	+	+/-
#49	+	+
#10	+	-
#46	+	+/-

Example 2

Purification of anti-PAR2 Hybridoma Antibodies for Screening

Hybridoma cells are cultured for a time and under conditions to yield a sample of about 35 ml of hybridoma supernatant fluid. To each sample is added 12 ml of 4×-Protein A Binding Buffer (1.6 M citric acid, 100 mM tris, pH 9.15) and about 300 μl of a 67% slurry of MabSelect™ Media (GE Healthcare, Piscataway, N.J.). The resulting slurry is rotated gently over night at 4° C.

After overnight incubation, the samples are centrifuged to sediment the resin and the monoclonal antibodies bound thereto, for example at 2,000 RPM in a G3.8 centrifuge rotor (Beckman Coulter, Fullerton, Calif.) for 5 minutes at 4° C. with no brake. All but about 300 μl of the supernatant fluid is removed and the resin is resuspended to form a concentrated slurry.

The concentrated slurry is transferred to a microcentrifuge tube and sufficient 1×-Protein A Binding Buffer (400 mM citric acid, 25 mM tris, pH 8.9) is added to bring the total volume up to about 1 ml. The slurry is resuspended, then centrifuged at about 14,000 g for 5 seconds. The supernatant fluid is removed from the resulting pellet, which is washed a total of three times in a similar manner (i.e. by resuspending in about 1 ml of 1×-Protein A Binding Buffer, centrifuging, removing supernatant and resuspending in fresh buffer).

After three washes, the pellet is resuspended in 400 μl Elution Buffer (200 mM formic acid) and agitated for 10 min at room temperature, then centrifuged at 14,000 g for 5 seconds. The supernatant is carefully removed as eluate, and the pellet is eluted again in a manner similar to that described above for a total of three elution cycles. The eluates from the three elution cycles are combined, centrifuge at 14,000 g for 5 min room temperature and transferred to a fresh tube. The pH is adjusted to 7.8-8.2 by adding 2 M tris base (235 mM) and mixing quickly. The samples are again centrifuged at 14,000 g for 5 min at room temperature, and designated as pH Shift Soluble. A spectral scan of each sample (diluted by adding 20 μl of the sample to 700 μl water) is run from 250 to 350 nm, and protein concentration is verified by loading 0.5 μg each antibody-containing sample on a reducing 4-20% SDS-PAGE gel with an appropriate antibody standard.

32

Example 3

Purification and Western Blot of PAR-2/Fc Polypeptide

Full length N-terminal PAR-2/Fc polypeptide (SEQ ID NO:5) is expressed in CHO cells. Expression supernatant from CHO expression cells cultured in serum-free media contain a CHO cell trypsin-like serine protease that cleaves PAR-2/Fc at the activation Arg-Ser bond, generating the “clipped” version of the PAR-2/Fc polypeptide. CHO expression cells cultured in 10% fetal calf serum (which contains normal levels of plasma proteinase inhibitors at concentrations far in excess of the concentration of the CHO cell trypsin-like serine protease) express full length N-terminal PAR-2/Fc in culture supernatants. Both clipped and full length proteins are purified using MabSelect™ resin substantially as previously described (see Example 2). The resultant purified Fc-constructs are analyzed by amino terminal sequence analysis (Edman degradation), size exclusion chromatography, absorbance spectral scan, and mass spectroscopy.

Various amounts of purified full length and clipped N-terminal PAR2-Fc are subjected to SDS-PAGE using 8-16% polyacrylamine gradient gels (Novex gels, Invitrogen Life Technologies) in a Tris-Glycine buffer system. Gel lanes containing See Blue standards (Novex, Invitrogen Life Technologies) for molecular weight identification are also included. Following electrophoresis, proteins are transferred from gels onto nitrocellulose membranes using a Novex XCell II Blot Module (Invitrogen Life Technologies). Membranes are blocked with 1:1, Odyssey blocking buffer, OBB, (LI-COR Biosciences):TBS (Tris Buffer Saline) overnight at 4 C with shaking. Antibodies to be analyzed are diluted in 1:1 OBB:TBS at a desired final concentration for 1 hr at room temperature. Membranes are washed extensively with 0.1% Tween 20 in TBS (3-4 changes of 100 ml over ~1 hr). Membranes are then exposed to the appropriate secondary antibody-Alexa680 (Molecular Probes, Invitrogen Life Technologies) conjugate (goat anti-rabbit IgG, or goat anti-mouse IgG) diluted 1:5000 in 1:1 (OBB:TBS) for 1 hr at room temperature. Membranes are washed as described above, and if desired, analyzed using a LI-COR Odyssey Infrared Imaging System (LI-COR Biosciences).

Example 4

Comparison of PAR-2 Antibodies

Several PAR-2 antibodies were tested in different assay formats; Table 2 summarizes the results.

TABLE 2

Clone ID #	IC ₅₀ (pre-cloning)	Binding to Cleavage site	
		Up stream	Down stream
47	8 nM	xxx	—
49	35 nM	xx	—
6	40 nM	xx	—
13	50 nM	xx	—
15	50 nM	xx	—
48	60 nM	xx	—
10	>1 mM	xx	xxx
46	>0.5 mM	xxx	xx

33

IC₅₀ values were determined in a FLIPR assay for Ca²⁺ mobilization described previously; binding to the upstream region of the cleavage site versus the downstream region of the cleavage site was determined using the Western blot assay described previously. More detailed results for a selected antibody are shown in FIG. 1. FIG. 2 presents Western blot results in which various antibodies were analyzed by Western blot against the cleaved (denoted 'clip') PAR-2/Fc and the full-length (denoted 'FL') PAR-2/Fc. FIG. 3 presents additional Western blot results, and also compares the characteristics of two monoclonal antibodies that present a contrast in recognition of full-length versus clipped PAR-2/Fc, which as detailed on the slide corresponds with ability to antagonize PAR-2. Thus, Clone 10 was weakly antagonistic in a flash plate assay, bound to cells expressing human PAR-2 (HCT116 cells), and to PAR-2-transfected CHO cells via FACS, but not to CHO parental cells, and bound to both clipped and full-length versions of PAR-2/Fc by Western blot. In contrast, Clone 33 was strongly antagonistic in a flash plate assay, bound to cells expressing human PAR-2 (HCT116 cells), and to PAR-2-transfected CHO cells via FACS, but not to CHO parental cells, and bound to the full-length version of PAR-2/Fc only by Western blot.

Example 5

Comparison of PAR-2 Antibody Subclones

Several PAR-2 antibodies were subcloned and tested for the ability to inhibit trypsin-induced PAR-2 activation; Table 2 summarizes the results.

34

TABLE 3

Clone ID #	IC ₅₀ HCT-116	IC ₅₀ KNRK	
5	13-8 prep 1	8 nM	471 nM
	13-8 prep 2	12 nM	188 nM
	6-6	3 nM	1556 nM
	21-5	8 nM	340 nM
	47.6	2 nM	254 nM
	47.7	2 nM	48 nM
10	49.2	5M	152 nM
	SAM11	>2 microM	>2 microM

IC₅₀ values were determined in a FLIPR assay monitoring Ca²⁺ mobilization as described previously, using either cells endogenously expressing human PAR-2 (HCT-116) or stably-transfected rat KNRK cells expressing PAR-2 (KNRK-PAR2). Two different purification preparations of subclone 13-8 were used; one (prep #1) was cultured and purified on a larger scale than previously described, but in substantially the same manner. SAM11 is a commercially available monoclonal antibody to PAR-2 (Santa Cruz Biotechnology, Inc., Santa Cruz, Calif.; U.S.A.) raised against the tethered ligand sequence of human PAR-2. In Western blots using the clipped and full-length forms of PAR-2 (described previously), SAM11 bound to both clipped and full-length versions of PAR-2/Fc, confirming that the SAM11 antibody epitope is downstream of the protease cleavage site. The results of a similar experiment using HCT-116 cells are shown in FIG. 4. Also included in this experiment is a polyclonal, monospecific antiserum raised against the loop 1 16-mer peptide described in Example 1.

Each reference cited herein is incorporated by reference in its entirety for all that it teaches and for all purposes.

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Ala Ala Ser Leu Ser Cys Ser Gly Thr Ile Gln Gly Thr Asn Arg Ser
20 25 30

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Ser Lys Gly Arg Ser Leu Ile Gly Lys Val Asp Gly Thr Ser His Val
35 40 45

act gga aaa gga gtt aca gtt gaa aca gtc ttt tct gtg gat gag ttt 192
Thr Gly Lys Gly Val Thr Val Glu Thr Val Phe Ser Val Asp Glu Phe
50 55 60

tct gca tct gtc ctc act gga aaa ctg acc act gtc ttc ctt cca att 240
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65 70 75 80

gtc tac aca att gtg ttt gtg gtg ggt ttg cca agt aac ggc atg gcc 288
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85 90 95

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Tyr Met Ala Asn Leu Ala Leu Ala Asp Leu Leu Ser Val Ile Trp Phe	
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ccc ttg aag att gcc tat cac ata cat ggc aac aac tgg att tat ggg	432
Pro Leu Lys Ile Ala Tyr His Ile His Gly Asn Asn Trp Ile Tyr Gly	
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gaa gct ctt tgt aat gtg ctt att ggc ttt ttc tat ggc aac atg tac	480
Glu Ala Leu Cys Asn Val Leu Ile Gly Phe Phe Tyr Gly Asn Met Tyr	
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Cys Ser Ile Leu Phe Met Thr Cys Leu Ser Val Gln Arg Tyr Trp Val	
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atc gtg aac ccc atg ggg cac tcc agg aag aag gca aac att gcc att	576
Ile Val Asn Pro Met Gly His Ser Arg Lys Lys Ala Asn Ile Ala Ile	
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ggc atc tcc ctg gca ata tgg ctg ctg att ctg ctg gtc acc atc cct	624
Gly Ile Ser Leu Ala Ile Trp Leu Leu Ile Leu Leu Val Thr Ile Pro	
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Leu Tyr Val Val Lys Gln Thr Ile Phe Ile Pro Ala Leu Asn Ile Thr	
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Thr Cys His Asp Val Leu Pro Glu Gln Leu Leu Val Gly Asp Met Phe	
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Asn Tyr Phe Leu Ser Leu Ala Ile Gly Val Phe Leu Phe Pro Ala Phe	
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Ala Met Asp Glu Asn Ser Glu Lys Lys Arg Lys Arg Ala Ile Lys Leu	
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Ile Val Thr Val Leu Ala Met Tyr Leu Ile Cys Phe Thr Pro Ser Asn	
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His Val Tyr Ala Leu Tyr Ile Val Ala Leu Cys Leu Ser Thr Leu Asn	
325 330 335	
agc tgc atc gac ccc ttt gtc tat tac ttt gtt tca cat gat ttc agg	1056
Ser Cys Ile Asp Pro Phe Val Tyr Tyr Phe Val Ser His Asp Phe Arg	
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gat cat gca aag aac gct ctc ctt tgc cga agt gtc cgc act gta aag	1104
Asp His Ala Lys Asn Ala Leu Leu Cys Arg Ser Val Arg Thr Val Lys	
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 85 90 95
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 His Val Tyr Ala Leu Tyr Ile Val Ala Leu Cys Leu Ser Thr Leu Asn
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 Ser Cys Ile Asp Pro Phe Val Tyr Tyr Phe Val Ser His Asp Phe Arg
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 Met Ala Leu Trp Ile Phe Leu Phe Arg Thr Lys Lys Lys His Pro Ala
 100 105 110
 Val Ile Tyr Met Ala Asn Leu Ala Leu Ala Asp Leu Leu Ser Val Ile
 115 120 125
 Trp Phe Pro Leu Ala Ile Ala Tyr His Leu His Gly Asn Asn Trp Val
 130 135 140
 Tyr Gly Glu Ala Leu Cys Lys Val Leu Ile Gly Phe Phe Tyr Gly Asn
 145 150 155 160
 Met Tyr Cys Ser Ile Leu Phe Met Thr Cys Leu Ser Val Gln Arg Tyr
 165 170 175
 Trp Val Ile Val Asn Pro Met Gly His Pro Arg Lys Lys Ala Asn Ile
 180 185 190
 Ala Val Gly Val Ser Leu Ala Ile Trp Leu Leu Ile Phe Leu Val Thr
 195 200 205
 Ile Pro Leu Tyr Val Met Lys Gln Thr Ile Tyr Ile Pro Ala Leu Asn
 210 215 220
 Ile Thr Thr Cys His Asp Val Leu Pro Glu Glu Val Leu Val Gly Asp
 225 230 235 240
 Met Phe Asn Tyr Phe Leu Ser Leu Ala Ile Gly Val Phe Leu Phe Pro
 245 250 255
 Ala Ile Leu Thr Ala Ser Ala Tyr Val Leu Met Ile Lys Thr Leu Arg
 260 265 270
 Ser Ser Ala Met Asp Glu His Ser Glu Lys Lys Arg Gln Arg Ala Ile
 275 280 285
 Arg Leu Ile Ile Thr Val Leu Ala Met Tyr Phe Ile Cys Phe Ala Pro
 290 295 300
 Ser Asn Leu Leu Leu Val Val His Tyr Phe Leu Ile Lys Thr Gln Arg
 305 310 315 320
 Gln Ser His Val Tyr Ala Leu Tyr Leu Val Ala Leu Cys Leu Ser Thr
 325 330 335
 Leu Asn Ser Cys Ile Asp Pro Phe Val Tyr Tyr Phe Val Ser Lys Asp
 340 345 350
 Phe Arg Asp His Ala Arg Asn Ala Leu Leu Cys Arg Ser Val Arg Thr
 355 360 365
 Val Asn Arg Met Gln Ile Ser Leu Ser Ser Asn Lys Phe Ser Arg Lys
 370 375 380

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Ser Gly Ser Tyr Ser Ser Ser Ser Thr Ser Val Lys Thr Ser Tyr
 385 390 395

<210> SEQ ID NO 4
 <211> LENGTH: 397
 <212> TYPE: PRT
 <213> ORGANISM: Rattus rattus

<400> SEQUENCE: 4

Met Arg Ser Leu Ser Leu Ala Trp Leu Leu Gly Gly Ile Thr Leu Leu
 1 5 10 15

Ala Ala Ser Ala Ser Cys Asn Arg Thr Val Asn Ala Pro Gly Pro Asn
 20 25 30

Ser Lys Gly Arg Ser Leu Ile Gly Arg Leu Asp Thr Pro Pro Pro Ile
 35 40 45

Thr Gly Lys Gly Ala Pro Val Glu Pro Gly Phe Ser Val Asp Glu Phe
 50 55 60

Ser Ala Ser Val Leu Thr Gly Lys Leu Thr Thr Val Phe Leu Pro Val
 65 70 75 80

Ile Tyr Ile Ile Val Phe Val Ile Gly Leu Pro Ser Asn Gly Met Ala
 85 90 95

Leu Trp Val Phe Phe Phe Arg Thr Lys Lys Lys His Pro Ala Val Ile
 100 105 110

Tyr Met Ala Asn Leu Ala Leu Ala Asp Leu Leu Ser Val Ile Trp Phe
 115 120 125

Pro Leu Lys Ile Ser Tyr His Leu His Gly Asn Asp Trp Thr Tyr Gly
 130 135 140

Asp Ala Leu Cys Lys Val Leu Ile Gly Phe Phe Tyr Gly Asn Met Tyr
 145 150 155 160

Cys Ser Ile Leu Phe Met Thr Cys Leu Ser Val Gln Arg Tyr Trp Val
 165 170 175

Ile Val Asn Pro Met Gly His Ser Arg Lys Arg Ala Asn Ile Ala Val
 180 185 190

Gly Val Ser Leu Ala Ile Trp Leu Leu Ile Phe Leu Val Thr Ile Pro
 195 200 205

Leu Tyr Val Met Arg Gln Thr Ile Tyr Ile Pro Ala Leu Asn Ile Thr
 210 215 220

Thr Cys His Asp Val Leu Pro Glu Glu Val Leu Val Gly Asp Met Phe
 225 230 235 240

Ser Tyr Phe Leu Ser Leu Ala Ile Gly Val Phe Leu Phe Pro Ala Leu
 245 250 255

Leu Thr Ala Ser Ala Tyr Val Leu Met Ile Lys Thr Leu Arg Ser Ser
 260 265 270

Ala Met Asp Glu His Ser Glu Lys Lys Arg Arg Arg Ala Ile Arg Leu
 275 280 285

Ile Ile Thr Val Leu Ser Met Tyr Phe Ile Cys Phe Ala Pro Ser Asn
 290 295 300

Val Leu Leu Val Val His Tyr Phe Leu Ile Lys Ser Gln Arg Gln Ser
 305 310 315 320

His Val Tyr Ala Leu Tyr Leu Val Ala Leu Cys Leu Ser Thr Leu Asn
 325 330 335

Ser Cys Ile Asp Pro Phe Val Tyr Tyr Phe Val Ser Lys Asp Phe Arg
 340 345 350

Asp Gln Ala Arg Asn Ala Leu Leu Cys Arg Ser Val Arg Thr Val Lys
 355 360 365

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Arg Met Gln Ile Ser Leu Thr Ser Asn Lys Phe Ser Arg Lys Ser Ser
370 375 380

Ser Tyr Ser Ser Ser Ser Thr Ser Val Lys Thr Ser Tyr
385 390 395

<210> SEQ ID NO 5
<211> LENGTH: 903
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(900)

<400> SEQUENCE: 5

atg cgg agc ccc agc gcg gcg tgg ctg ctg ggg gcc gcc atc ctg cta 48
Met Arg Ser Pro Ser Ala Ala Trp Leu Leu Gly Ala Ala Ile Leu Leu
1 5 10 15

gca gcc tct ctc tcc tgc agt ggc acc atc caa gga acc aat aga tcc 96
Ala Ala Ser Leu Ser Cys Ser Gly Thr Ile Gln Gly Thr Asn Arg Ser
20 25 30

tct aaa gga aga agc ctt att ggt aag gtt gat ggc aca tcc cac gtc 144
Ser Lys Gly Arg Ser Leu Ile Gly Lys Val Asp Gly Thr Ser His Val
35 40 45

act gga aaa gga gtt aca gtt gaa aca gtc ttt tct gtg gat gag ttt 192
Thr Gly Lys Gly Val Thr Val Glu Thr Val Phe Ser Val Asp Glu Phe
50 55 60

tct gca tct gtc ctc act gga aaa gtc gac aaa act cac aca tgc cca 240
Ser Ala Ser Val Leu Thr Gly Lys Val Asp Lys Thr His Thr Cys Pro
65 70 75 80

ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc 288
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
85 90 95

ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc 336
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
100 105 110

aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc 384
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
115 120 125

aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg 432
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
130 135 140

cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc 480
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
145 150 155 160

gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc 528
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
165 170 175

tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc 576
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
180 185 190

aaa ggg cag ccc cga gag cca cag gtg tac acc ctg ccc cca tcc ccg 624
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
195 200 205

gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc 672
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
210 215 220

ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg 720
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
225 230 235 240

gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc 768
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
245 250 255

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ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag      816
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
      260                      265                      270

ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac      864
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
      275                      280                      285

tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga      903
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
      290                      295                      300

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<210> SEQ ID NO 6
<211> LENGTH: 300
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 6

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Met Arg Ser Pro Ser Ala Ala Trp Leu Leu Gly Ala Ala Ile Leu Leu
1          5          10          15

Ala Ala Ser Leu Ser Cys Ser Gly Thr Ile Gln Gly Thr Asn Arg Ser
20          25          30

Ser Lys Gly Arg Ser Leu Ile Gly Lys Val Asp Gly Thr Ser His Val
35          40          45

Thr Gly Lys Gly Val Thr Val Glu Thr Val Phe Ser Val Asp Glu Phe
50          55          60

Ser Ala Ser Val Leu Thr Gly Lys Val Asp Lys Thr His Thr Cys Pro
65          70          75          80

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
85          90          95

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
100         105         110

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
115        120        125

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
130        135        140

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
145        150        155        160

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
165        170        175

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
180        185        190

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
195        200        205

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
210        215        220

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
225        230        235        240

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
245        250        255

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
260        265        270

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
275        280        285

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
290        295        300

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<210> SEQ ID NO 7

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<211> LENGTH: 8
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Peptide

<400> SEQUENCE: 7

Asp Tyr Lys Asp Asp Asp Asp Lys
 1 5

What is claimed is:

1. A method of decreasing PAR-2 activity in a subject with an inflammatory condition of the skin, joints, gastrointestinal system and/or airway comprising administering to the subject a pharmaceutical comprising an antigen binding protein that binds to full-length proteinase activated receptor-2 (PAR-2) and binds to a lesser extent to cleaved PAR-2, thereby binding a loop 1 peptide of PAR-2 consisting of amino acids 29 through 46 of SEQ ID NO:2 which includes a proteolytic cleavage site between R36 and S37 upstream of the cleavage site, wherein the antigen binding protein antagonizes the proteolytic activation of PAR-2 with an IC50 of 60 nM or less in a fluorometric imaging plate reader (FLIPR) assay using HCT-116 cells.

2. The method of claim 1 wherein the PAR-2 activity that is decreased is PAR-2 signalling.

3. The method of claim 2, wherein the PAR-2 signalling decrease results from inhibition of proteolytic activation of PAR-2.

4. The method of claim 1 wherein the inflammatory condition is selected from the group consisting of asthma, and chronic obstructive pulmonary disease.

5. The method of claim 4, wherein the condition is asthma.

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